SYNTHESIS AND RADIOCHEMICAL STABILITY EVALUATION OF RADIOPHARMACEUTICAL COMPOUNDS CONTAINING RADIOIODINATED PROSTHETIC GROUPS

by

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DECLARATION

I the undersigned hereby declare that the work contained in this dissertation is my own original work and has not previously in its entirety or in part been submitted at any other university for a degree.

D.D. ROSSOUW

SUMMARY

A study was undertaken to investigate the radiochemical stability of the β-iodoethoxyl moiety, a relatively novel prosthetic group employed in radiopharmaceutical chemistry, in which an oxygen atom in a β-position relative to the radioiodine atom has a stabilising effect on the aliphatic carbon-iodine bond. The investigation was started as a pilot study by synthesising various model compounds containing a β-radioiodoethoxyl moiety, as well as two reference compounds lacking such a moiety. The purpose was to determine the influence of various groups in the vicinity of the β-oxygen atom on the stability of the abovementioned moiety. Radiochemical stability tests were carried out *in vitro* at 37°C in human blood serum. The results confirmed the superior stability of such a moiety compared to that of the reference compounds and also showed that the branching of such an aliphatic unit resulted in a considerable improvement in its stability, especially over a longer period.

The investigation was extended to the synthesis of other compounds containing a few selected β-iodoethoxyl moieties that showed improved stability in the pilot study work. Reference compounds containing the classical iodovinyl unit, as well as those lacking a stabilising β-oxygen atom, were also prepared. The carrier molecules used in this part of the work was a benzamide containing a phenolic oxygen atom which acted as the β-oxygen atom, as well as two heterocyclic amines, benzotriazole and 2-methyl-5-nitroimidazole, in which the β-iodoethoxyl moiety was linked to a secondary nitrogen atom. Various suitable alkylating agents were prepared, chemically linked to the carrier molecules, the resulting intermediate compounds converted into tosylate or triflate iodination precursors and labelled with radioiodine by means of iodide-for-tosylate/triflate exchange. *In vitro* stability tests of these compounds showed similar trends to those obtained with the model compounds. Moreover, the stability of the stabilised β-iodoethoxyl moiety compared favourably with that of the iodovinyl unit, especially when incorporated into a heterocyclic amine. The results of this study have

shown that some of the radioiodinated compounds synthesized in this work, especially the nitroimidazole derivatives, have the potential to be considered as novel radiopharmaceuticals.

OPSOMMING

'n Studie is onderneem om die radiochemiese stabiliteit van die β-jodium-etoksi-eenheid te ondersoek. Dié eenheid is 'n relatief nuwe prostetiese groep wat in radiofarmaseutiese chemie gebruik word. Die suurstofatoom wat in 'n β-posisie relatief tot die radiojodiumatoom voorkom, oefen 'n stabiliserende invloed op die alifatiese koolstof-jodiumbinding uit. Die ondersoek het met 'n loodsstudie begin deur verskillende modelverbindings te sintetiseer wat 'n β-radiojodium-etoksi-eenheid bevat, asook twee verwysingsverbindings waarin so 'n eenheid ontbreek. Die doel hiermee was om die invloed van verskillende groepe, wat in die omgewing van die β-suurstofatoom voorkom, op die stabiliteit van die eenheid te bepaal. Radiochemiese stabiliteitstoetse is uitgevoer deur middel van inkubering in menslike bloedserum by 37°C. Die resultate het die groter stabiliteit van so 'n eenheid in vergelyking met dié van die verwysingsverbindings aangetoon, en het ook uitgewys dat vertakking van so 'n alifatiese eenheid 'n aansienlike verbetering in die stabiliteit tot gevolg gehad het, veral oor 'n langer periode.

Die ondersoek is vervolgens uitgebrei deur verdere verbindings te sintetiseer wat beskik oor bepaalde uitgesoekte β-jodium-etoksi-eenhede, wat verbeterde stabiliteit in die loodsstudie getoon het. Verwysingsverbindings wat die klassieke jodiumvinieleenheid bevat het, sowel as dié waarin 'n stabiliserende β-suurstofatoom ontbreek het, is ook berei. Die draermolekules wat in hierdie deel van die studie gebruik is, was 'n bensamied met 'n fenoliese suurstofatoom wat as die β-suurstofatoom gedien het, sowel as twee heterosikliese amiene, bensotriasool en 2-metiel-5-nitroimidasool, waarin die β-jodium-etoksi-eenheid aan 'n sekondêre stikstofatoom geheg is. Verskillende geskikte alkileermiddels is berei, aan die draermolekules geheg, die tussenprodukte omskep in tosilate of triflate en met radiojodium gemerk deur middel van jodium-vir-tosilaat/triflaat-uitruiling. Stabiliteitstoetse van hierdie verbindings in bloedserum het soortgelyke tendense as dié van die aanvanklike modelverbindings getoon. Daarbenewens het die stabiliteit van die gestabiliseerde β-jodium-etoksi-eenheid gunstig vergelyk met dié van

die jodiumviniel-eenheid, veral wanneer dit deel gevorm het van 'n heterosikliese amien. Die resultate van die studie het getoon dat sommige van die radiogejodeerde verbindings wat berei is, veral die nitroimidasoolderivate, die potensiaal het om as nuwe radiofarmaseutiese verbindings gebruik te kan word.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

LIST OF ABBREVIATIONS

DMF	Dimethylformamide
GC	Gas Chromatography
HMPA	Hexamethylphosphoramide
HMPT	Hexamethylphosphorous triamide
HPLC	High Performance Liquid Chromatography
OTf	Trifluoromethanesulphonyloxy
OTs	*p*-Toluenesulphonyloxy
PET	Positron Emission Tomography
SPECT	Single Photon Emission Computed Tomography
TBS-PROP-TOS	[*E*]-3-(tri-*n*-butylstannyl)prop-2-en-1-yl *p*-toluenesulphonate
TET	Ethylene di-*p*-toluenesulphonate
TLC	Thin Layer Chromatography
TPT	Propylene 1,2-di-*p*-toluenesulphonate
p-TsCl	*p*-Toluenesulphonyl chloride

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 OVERVIEW OF RADIOPHARMACEUTICALS

1.1.1 Radiopharmacy: Definitions and History

Radiopharmaceuticals can be defined as radioactive nuclide-containing drugs that are used in nuclear medicine for noninvasive diagnostic organ or tumour imaging, metabolism studies or therapy. The purpose for which it is used depends on the energy of the radiation emitted by the radionuclide. The radionuclide acts as the "active ingredient", while the drug or organic compound to which it is attached merely acts as a vehicle or carrier that delivers the radionuclide to its biological target. Diagnostic imaging is carried out using gamma-emitting nuclides in the energy range of 150 to 300 keV, and a sophisticated scanning module called a gamma camera. These cameras are equipped with large sodium iodide-crystal detectors with which the emitted gamma photons interact. All data are recorded simultaneously and continuously, resulting in well-defined images of the organ or tumour.

Radiopharmacy is the science and art of preparing and dispensing these radiopharmaceuticals, while radiopharmacology refers to their utilisation for demonstrating their distribution, deposition, kinetics of metabolism, turnover, and their final excretion from the body. The practice of radiopharmacy as a specialised medical tool began early in the twentieth century. It all perhaps started in consequence of a report on the injections of radium into the skin lesions of lupus (*Wickham & Degrais, 1911*), followed shortly afterwards by another report on the intravenous injection of radium for the therapy of various diseases (*Proescher, 1914*). In 1928 a natural radionuclide, a preparation from thorium dioxide, was introduced in Germany as an intravenously administered contrast medium for diagnostic radiology (*Casper, 1967*). The

artificial production of radionuclides began in the early 1930s. In 1934, E.O. Lawrence, the inventor of the cyclotron, first produced radioactive nuclides by bombarding stable atoms with artificially accelerated particles (*Lawrence, 1965*). His brother, J.H. Lawrence, made the first clinical therapeutic application of an artificial radionuclide when he used ^{32}P to treat leukemia in 1937 (*Lawrence, 1940*). In 1946, radionuclides such as ^{14}C (as barium carbonate), which were produced in the Oak Ridge reactor in Tennessee, USA, were made available for biological and medical purposes (*Andrews, 1976*).

The late 1940s and early 1950s saw the beginning of rapid growth in the medical applications of radionuclides. Before this stage, most radionuclides had been used in rather simple chemical forms. In some cases, the nuclides had been attached as labels to molecules already familiar in their stable form. Relatively little was done with regard to creating entirely new labelled compounds. In 1946, Abbott Laboratories started to develop and produce radioactively labelled compounds for medical use. One of the first radioiodine-labelled compounds reported was ^{131}I-labelled diiodofluorescein (*Andrews, 1976*), which was used for the detection of brain tumours, using Geiger-Müller tubes that were placed on the scalp of the patient, as detection device. This very primitive type of detection apparatus was soon replaced by more modern scanners, and eventually by the gamma camera. The emergence of other radioiodine-labelled radiopharmaceuticals, such as serum albumin, antibodies and quinoline derivatives, soon followed. Today, a vast number of established radiopharmaceutical compounds is available, and extensive research is still continuing in order to develop improved products.

1.1.2 Design and Synthesis of Radiopharmaceuticals

Radiopharmaceuticals are designed according to the same principles that are applied to the development of non-radioactive pharmaceuticals. One of these principles is the all-important relationship between structure and activity. The affinity of a radiopharmaceutical for a specific biological target depends largely on the structure of the carrier. The radiopharmaceutical usually binds to a specific organ or tumour in the body through interaction with a receptor.

However, binding can also take place at so-called non-receptor sites, resulting in errors in receptor binding measurements (*Katzenellenbogen et al., 1981*). The optimal design of a radiopharmaceutical is therefore of critical importance in order to obtain images of high quality that will provide the correct information. In this thesis, structure-activity relationships will not be discussed in further detail, as this issue was deemed to be beyond the scope of the present research project.

When designing new radiopharmaceuticals, other factors should also be taken into consideration. The choice of radionuclide, as well as its physical properties such as mode of decay and energy associated with it, is also crucial with regard to the intended application of the radiopharmaceutical. Modern radiopharmaceuticals are classified into two types, namely so-called SPECT (single photon emission computed tomography) and PET (positron emission tomography) tracers. SPECT tracers contain medium- to relatively long-lived radionuclides with predominant gamma emission and are the most general tracers used in nuclear medicine. PET tracers contain mostly accelerator-produced short-lived positron emitters, are generally more expensive than SPECT tracers, and require much more sophisticated scanning equipment for diagnostic imaging. Positron emitters such as ^{18}F and ^{11}C are incorporated in molecules designed for PET tracers, and gamma emitters such as ^{123}I, ^{131}I and ^{99m}Tc in SPECT tracers. Due to its more favourable decay characteristics and principal gamma-ray energy of 159 keV, ^{123}I is predominantly used in diagnosis, while ^{131}I with its higher principal gamma energy of 364.5 keV is mostly used in therapy and to a lesser extent in diagnosis.

An organic radiopharmaceutical is produced by incorporating a radionuclide into pharmaceuticals, biochemical substrates and metabolic intermediates. This process is generally carried out by substituting a stable atom or leaving group in an organic compound with a radionuclide, and is called radiolabelling or labelling. The radionuclide can be an isotope of the substituted atom, or it can be a completely different atom. For example, a stable carbon atom can be substituted with its radioisotopes ^{11}C or ^{14}C. A stable hydrogen atom can likewise be substituted with a ^{3}H atom (tritium labelling), or with a radioactive iodine (^{123}I, ^{131}I) or fluorine

(^{18}F) atom. Another way to incorporate a radionuclide into an organic structure is by means of complex formation. This type of labelling process is usually utilised for incorporating radiometals into organic compounds. Technetium- and gallium-labelled compounds are produced this way. The radionuclide ^{99m}Tc is obtained from a $^{99}Mo/^{99m}Tc$ generator system as the pertechnetate anion. In this chemical form it is in its highest oxidation state. A prerequisite for complex formation is the lowering of the oxidation state of the metal. In order to carry out a labelling process, the generator eluate is simply mixed with a suitable ligand in the presence of a suitable chemical reductant at room temperature. Such ligands are usually marketed as so-called "cold[1] kits", and are kept in sealed, aseptic glass vials in a lyophilised form. Today, ^{99m}Tc-labelled compounds are the most frequently used SPECT tracers in nuclear medicine due to their relative ease of preparation, as well as the ready availability and relatively low cost of the isotope.

For the purpose of this study, emphasis will be placed solely on diagnostic type of radiopharmaceuticals containing radioactive iodine. There is a wide range of radioiodinated radiopharmaceuticals due to the relative ease with which iodine can react with many organic compounds. Examples of these are the following: radioiodinated benzylguanidine for the imaging of tumours associated with the adrenal glands (*McEwan et al., 1986*), radioiodinated hippuric acid or long-chain fatty acids for kidney or heart muscle imaging respectively (*Zielinski et al., 1977*; *Machulla et al., 1980b*) and radioiodinated benzamides for brain imaging (*Kung et al., 1988a*).

The most commonly used radiolabelling methods employed for the production of radioiodinated compounds are the following:

(1) Exchange of a stable iodine atom in the molecule with a radioactive iodine atom (isotope exchange).

(2) Exchange of a stable chlorine or bromine atom in the molecule with radioiodine (halogen exchange in a so-called Finkelstein substitution).

[1] The term "cold" refers to non-radioactive compounds

(3) Substitution of a stable hydrogen atom with radioiodine (direct iodination).
(4) Substitution of a good leaving group, pre-introduced into the molecule, with radioiodine.

The exchange reactions, as well as the leaving group substitution, can be carried out on aryl-X as well as on alkyl-X compounds, where X is the atom to be exchanged or substituted. Direct iodination is limited to aryl-X compounds only, which requires activation of the aromatic ring towards electrophilic substitution.

All of these methods result in products with different compositions. The isotope exchange reactions produce products that have so-called low specific activities. The term specific activity is defined as the activity per unit mass of an element or compound containing the radioactive nuclide. It can also be said that the mass or molar ratio between the radioiodinated compound and its stable precursor[2] is low due to the small percentage of stable atoms that is exchanged. The mass associated with the radioactivity is only in the order of nano- to picograms, while the mass of the precursor is in the order of milli- to micrograms. In isotope exchange reactions the radiolabelled compound obviously cannot be separated from its stable precursor because of their identical chemical properties. The other radiolabelling methods, on the other hand, can lead to products with higher specific activities. For example, when a bromine atom is exchanged with a radioiodine atom in the Finkelstein reaction, the radioiodinated species can be fairly well separated from its non-radioactive brominated precursor by chromatographic means. The substitution reactions invariably result in radiopharmaceuticals with much higher effective specific activities, as the radioiodinated species can be quite effectively separated from their respective precursors. Having the precursor as an impurity in a radioligand would, on the other hand, reduce the effective specific activity of the radiopharmaceutical (*Pike et al., 1991*). The specific activity of radiopharmaceuticals is important, especially in receptor imaging. If the specific activity is too low, the receptor can easily be saturated with stable precursor (*Pike et al., 1991*), resulting in the exclusion of the radiopharmaceutical. This

[2] A precursor is the starting material used for the synthesis of a radioiodinated compound

prerequisite does not apply to all types of radiopharmaceuticals, and the isotope exchange method is therefore fairly commonly used in radiochemistry.

To summarise, the chemical structure of a chosen target molecule (i.e. the molecule to be radiolabelled) must not only be in line with structure-activity requirements, but must also allow relatively fast labelling, which would result in high radiochemical yield and, when needed, high specific activity. Other important requirements regarding the design of radiopharmaceuticals will be discussed in the following paragraph.

1.1.3 Radiochemical Stability of Radiopharmaceuticals

The properties of a radiopharmaceutical are not only important with regard to its efficiency of binding to a receptor or organ, but also with regard to its radiochemical stability. A radiopharmaceutical is of no use if it cannot retain its radioactive nuclide, as the biological pathway of the unbound nuclide is completely different to that of the covalently bound nuclide. For example, free radioiodine will accumulate in the thyroid, while radioiodinated hippuric acid will migrate to and be excreted by the kidneys. Deiodination can therefore result in unwanted radiation of healthy organs (*Warren et al., 1990*). The radioiodine should therefore be introduced regioselectively into an organic compound to ensure optimum stability of the covalent carbon-iodine bond. However, the relatively poor biochemical stability of the C-I bond often leads to dehalogenation and a high blood background which could seriously affect imaging (*Coenen et al., 1983*).

Deiodination of radioiodinated compounds can occur "*in vitro*" (outside the body), as well as "*in vivo*" (inside the body). Storage of such compounds in solution at room or elevated temperatures might result in the gradual decomposition of the carbon-iodine bond due to radiolysis, a process that accompanies the radioactive decay of the radionuclide (*Verbruggen, 1987*; *Chattopadhyay et al., 2001*). Radiolysis is mostly pronounced at high activity levels (more than 225 MBq/ml) (*Eersels & Herscheid, 2001*) and when dealing with α-emitting

nuclides or nuclides which decay during processes followed by an Auger process and the emission of a high number of electrons (*Coenen et al., 1983*). Radiopharmaceuticals with high specific activities can also exhibit radiolytic decomposition (*Coenen et al., 1983*). This process can be diminished by the addition of radical absorbers and bactericidal agents such as benzyl alcohol, *para*-hydroxybenzoic acid, *etc.* (*Saha, 1983*; *Wafelman et al., 1994*). The stability of radiohalogenated pharmaceuticals (tracers) under physiological conditions is very important for their *in vivo* application. *In vivo* deiodination is probably of bigger concern to nuclear pharmacists because the breakdown of the molecule cannot be controlled. The deiodination of radioiodinated antibodies can be caused by three processes (*Warren et al., 1990*), namely by hydrolytic, enzymatic (*Eary et al., 1988*) and catabolic biochemical means (*Leninger, 1982*). These deiodination processes can be limited by introducing various structural changes to compounds. Warren *et al.* postulated that hydrolytic deiodination can be minimised by employing stronger aryl-iodine bonds, while enzymatic deiodination could be inhibited by the elimination of adjacent hydroxyl groups in aromatic compounds. The presence of a hydroxyl group in the aromatic ring of a radioiodinated antibody *ortho* to an iodine atom can also lead to some *in vivo* deiodination (*Vaidyanathan et al., 1995*). Krummeich *et al. (1994)* claimed that *O*-methylation, as well as methylation in the alpha-position of tyrosine, increase the *in vivo* stability of the radioiodinated product towards deiodination.

The chemical as well as physical nature of carbon-iodine bonds, for example, the C-I bond energy, are important factors regarding the stabilities of radioiodinated tracers (*Coenen et al., 1983*). The bond energy of aryl halides is generally 40 to 80 kJ/mole higher than that of alkyl halides, while vinyl iodides exhibit slightly higher C-I bond energies than aryl iodides. Furthermore, halogens on aromatic rings are usually metabolically stable, and vinyl compounds are more stable *in vivo* than aliphatic ones (*Chenoweth & McCarty, 1963*). Generally, compounds having sp^2 carbon-bonded iodine have a higher metabolic stability than those with iodoalkyl functionalities (*Seevers & Counsell, 1982*; *Musachio & Lever, 1992*). The chemical environment surrounding the labelled tracer is also important. Mazaitis *et al.*, (1980) have, for example, found that iodoethynyl derivatives are stable under synthetic conditions but were

completely deiodinated in blood plasma within one hour. ω-Halogenated long-chain fatty acids show no decomposition when stored for one hour in blood (*Kupfernagel, 1978*), while half of the bonded iodine is released from 17-[^{123}I]-iodoheptadecanoic acid in the heart muscle cells of mice 30 seconds after i.v. injection (*Kupfernagel, 1978*; *Stöcklin et al., 1980*). This implies that the metabolic process which degrades the iodinated fatty acid is relatively fast (*Kloster & Stöcklin, 1982*). On the other hand, the halogen in halophenyl fatty acids is metabolically stable (*Stöcklin et al., 1980*; *Machulla et al., 1980a*).

To summarise, there are numerous factors that can prevent a radiotracer from reaching its target inside the body. Not only can it lose its radionuclide before being administered, but this can also take place inside the body. Radiopharmaceuticals are normally administered as soon as possible after they are synthesised due to the relatively short half-lives of their incorporated radionuclides. For this reason, radiochemists are not too concerned about their shelf stability, as this, to a certain extent, can be controlled. Their biological breakdown poses a much bigger problem, and this has been the topic of extensive research.

The preceding arguments imply that the position of a radionuclide in a molecule, as well as the presence of neighbouring groups, largely influences the stability of the tracer. Ideally, radioiodine should be introduced into an aromatic moiety of a target molecule due to the relatively higher stability of the aromatic carbon-iodine bond (*Mennicke et al., 2000*). Direct electrophilic radioiodination is most commonly used, but many molecules do not possess aromatic rings or are not suited to this procedure because they lack activating functionality or lose biological activity when iodinated at the chemically favoured site (*Musachio & Lever, 1992*). For example, molecules like the neuroreceptor ligand diprenorphine (a 4,5-epoxymorphinan) contain a highly activated aromatic (phenolic) ring suitable for direct electrophilic radioiodination. However, direct substitution of the aromatic ring with heavy halogens leads to a substantial reduction in potency of 4,5-epoxymorphinans (*Casy & Parfitt, 1986*; *Wang et al., 1995*). For all these reasons, radiochemists have to make use of alternative

methods to introduce a radionuclide into an organic molecule. This will be discussed in the following section.

1.2 PROSTHETIC GROUPS IN RADIOPHARMACEUTICAL CHEMISTRY

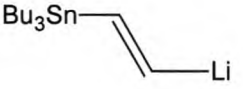
1.2.1 Introduction

It was mentioned in the previous section that the aromatic portion of a molecule is the most commonly used site for the introduction of radioiodine, but that, due to certain restrictions, the scope for this technique is limited. In order to circumvent this problem, radiochemists make use of so-called prosthetic groups that act as a "bridge" between the parent molecule (i.e. the unmodified molecule to be radiolabelled) and the radionuclide. Prosthetic groups usually are relatively small organic units that can easily be attached to the parent molecule without significantly changing its chemical, physical or biological properties. Prosthetic groups can also fulfil other functions, such as enhancing the reactivity of a molecule, improving its stability, controlling regiospecificity and preventing harsh labelling reaction conditions (*Musachio & Lever, 1992*). The reagents used for the introduction of prosthetic groups into radiopharmaceuticals typically contain two functional groups, one for attaching the unit to the parent molecule via an established chemical method (e.g. nucleophilic addition), and the other to serve as a good leaving group that can be substituted by a suitable radionuclide (e.g. in radioiododestannylation reactions) (*Musachio & Lever, 1992*). In most cases the attachment step will precede the displacement step as it is advisable to incorporate the radionuclide in the final step of any synthesis. It also follows from previous arguments that the carbon-radionuclide bond in a prosthetic group should be very stable in order to prevent loss of the radionuclide.

1.2.2 Types of Prosthetic Groups

Various types of prosthetic groups of varying size, have been used. One classical example is the so-called Bolton-Hunter reagent, a radioiodinated 3-(*p*-hydroxyphenyl)propionic acid *N*-

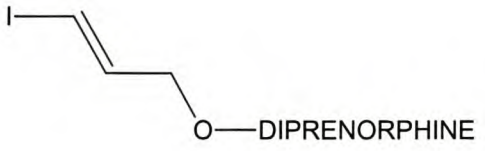
hydroxysuccimide ester, tailored for conjugation to proteins, antibodies and glycocides (*Musachio & Lever, 1992*; *Bolton & Hunter, 1973*; *Lowndes et al., 1988*; *Wilbur et al., 1989*; *Lin et al., 1989*; *Vaidyanathan & Zalutsky, 1990*). However, due to its relatively large size, it is of limited applicability to small molecules (*Musachio & Lever, 1992*). A vast number of other radiolabelled reagents has been used for protein conjugation and has been reviewed by Wilbur (1992). These include activated esters, imidate esters, aldehydes, isocyanates and isothiocyanates. All of these reagents react with protein amines to form different types of bond linkages (amides, amidines, imines, ureas and thioureas). *para*-Iodobenzylbromide is one of the few prosthetic groups chiefly intended for radiolabelling small molecules with either ^{125}I or ^{123}I (*Wilson et al., 1986*), but it has also been applied to the synthesis of radioiodinated glucose analogues (*Saji et al., 1987*). This reagent is particularly appropriate when the benzyl unit is an integral part of the target (i.e. the molecule to be radiolabelled) (*Musachio & Lever, 1992*). For radioiodination of small molecules, prosthetic groups, which impart less bulk and lipophilicity, are desired (*Musachio & Lever, 1992*). The lithiated vinylstannane **1.1** is the smallest prosthetic group that has been used in radioiodinations (*Musachio & Lever, 1992*).



1.1

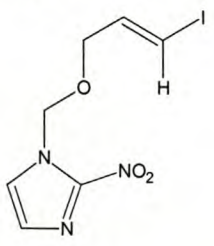
After nucleophilic addition to carbonyl groups and subsequent halodemetalation this group yields iodovinyl derivatives (*Hanson & Seitz, 1982*; *Hanson, Seitz & Botarro, 1982*). (*E*)- and (*Z*)-3-(Tributylstannyl)prop-2-en-1-yl tosylates are other allylic bifunctional alkylating agents which render vinylstannanes for radioiodination (*Musachio & Lever, 1989*). The relatively high stability of vinyl iodides has already been referred to. These bifunctional agents can be attached to an OH-group in a target molecule such as diprenorphine via a coupling reaction involving the tosylate group, followed by an electrophilic radioiododestannylation reaction, involving the tributylstannyl group, to produce *O*-iodoallyl compounds with the oxygen atom in a gamma position relative to the iodine (*Musachio & Lever, 1992*; *Wang et al., 1995*). This type of prosthetic group is present in compound **1.2**. The derivatisation of the OH group with an

alkynylic halide such as propargyl bromide, followed by hydrostannylation of the triple bond and subsequent radioiododestannylation is an alternative synthetic route towards these compounds (*Van Dort et al., 1999*).

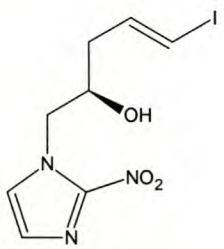


1.2

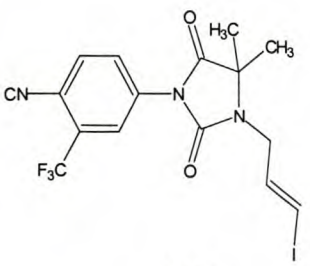
Secondary amino groups in molecules can also be functionalised to produce *N*-iodoallyl compounds (*Chumpradit et al., 1995*; *Waterhouse et al., 1996*; *Waterhouse et al., 1998*). The synthesis of an ^{125}I-labelled 2-nitroimidazole derivative is an interesting example of the introduction of an oxygen atom into an iodoallylic prosthetic group in a gamma position relative to the iodine (*Hasan et al., 1991*). The propargyloxymethylation of 2-nitroimidazole was followed by hydrostannylation and radioiododestannylation as previously described, to produce the N-CH_2O-iodoallyl compound (**1.3**). Another example featuring this type of prosthetic group, is the (iodovinyl)misonidazole derivative (*E*)-5-(2-nitroimidazolyl)-4-hydroxy-1-iodopent-1-ene (**1.4**) (*Biskupiak et al., 1991*). The nitrogen atom in a cyclic amide structure such as hydantoin has also been functionalised with an iodoallyl group (**1.5**) (*Van Dort & Hagen, 2001*). The allylic bifunctional alkylating agent in this case was (*E*)-1-chloro-3-(tri-*n*-butylstannyl)-2-propene. Iodovinyl groups can also be attached to a carbon atom that forms part of a cyclic structure to produce iodovinyl derivatives. The radioiodine-labelled iodovinylestradiol derivatives **1.6a** & **b** (*Hanson, Seitz & Botarro, 1982*; *Kabalka et al., 1991*; *Napolitano et al., 1995*) and iodovinyltetrabenazine (**1.7**) (*Canney et al., 1993*) are examples of such compounds.



1.3



1.4



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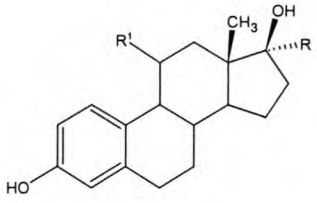
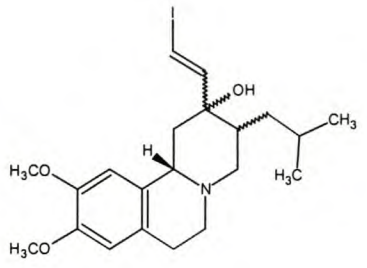


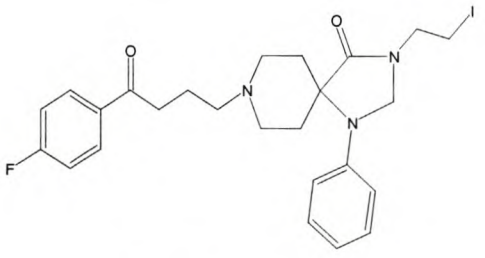


R^1=H; R= -$(CH_2)_x$-CH=CH-I (x = 0 - 8) **1.6a**
R^1= -CH=CH-I; R=H **1.6b**

1.7

Aliphatic prosthetic groups containing either radioiodine or radiofluorine but lacking the stabilising effect of a vinyl bond or other groups have also been described. The alkylation of secondary amines or amides with fluoroalkyl groups has been well documented, and a few similar iodoalkyl derivatives have also been reported. In the synthesis of [^{125}I]iodoethylspiperone, a radioligand used for the exploration of central dopamine receptors in the brain, the iodoethyl group is introduced at the lactam nitrogen atom of spiperone (**1.8**) (*Chalon et al., 1990*). Surprisingly, this compound exhibited good *in vivo* stability in rats, which was indicated by the slow uptake of radioactivity in the thyroid. No explanation for this unusual stability of the C-I bond is given, but it is possible that the nearby lactam carbonyl group could have a stabilising effect. A similar ^{18}F-labelled spiperone derivative, [^{18}F]fluoroethylspiperone, was also documented (*Kiesewetter et al., 1986*; *Chi et al., 1986*). Other compounds containing the *N*-fluoroalkyl unit are 1-(2-fluoroethyl)-2-nitroimidazole and 1-(2-fluoroethyl)-2-methyl-5-nitroimidazole (**1.9**) (*Jerabek et al., 1986*), [^{18}F]fluororaclopride (**1.10**) (*Kiesewetter et al., 1989*), *N*-fluoropropyltetralin- (**1.11**) (*Zijlstra et al., 1993a*; *Shi et al., 1999*), *N*-fluoropropylapomorphine derivatives (*Zijlstra et al., 1993b*), ω-fluoroalkylated derivatives of 1-phenylpiperazine (**1.12**) (*Chi et al., 1986*) as well as *N*-fluoropropylnormetazocine- and *N*-fluoropropyllorazepam derivatives (*Shiue et al., 1986*). A few *C*-fluoroalkyl compounds such as 11β-(2-fluoroethyl)estradiol derivatives (*French et al., 1993*) and 4-fluoromethylcyclohexyl derivatives (*Windhorst et al., 1999*) have also been documented. Other examples of radioiodine-labelled compounds with the radionuclide in an apparently non-stabilised aliphatic position are ^{131}I-labelled ornidazole [1-iodo-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol (**1.13**) (*Aşikoğlu et al., 1998*), ^{125}I-labelled 5-(1-hydroxy-2-

iodoethyl)-2'-deoxyuridine (**1.14**) (*Iwashina et al., 1990*) and ^{131}I-labelled "Flagyl" [1-(2-iodoethyl)-2-methyl-5-nitroimidazole] (**1.15**) (*Tubis et al., 1975*). In the case of the labelled ornidazole, allegedly an inflammation or infection detecting agent (*Asikoglu et al., 2000*), the radioiodine is incorporated into a propan-2-ol unit, attached to the terminal carbon adjacent to the OH-bearing carbon. No information regarding radiochemical stability was given. However, positive results were reported in imaging and biodistribution studies in rabbits and rats respectively (*Asikoglu et al., 2000*). In the case of the labelled deoxyuridine, the radioiodine is present in the 2-iodoethanol unit, attached to a terminal carbon adjacent to an OH-bearing carbon similar to that in the ornidazole derivative. No radiochemical stability data or any biodistribution results for this compound were given.



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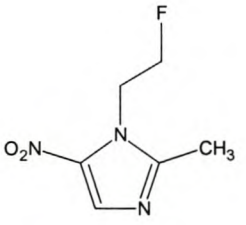

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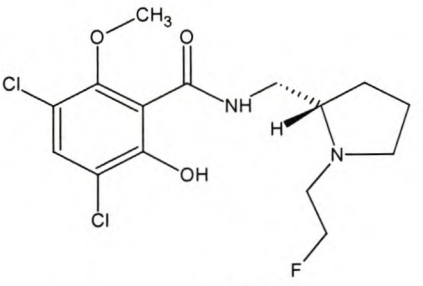

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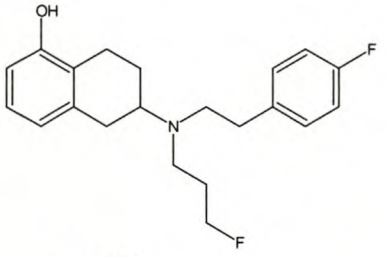

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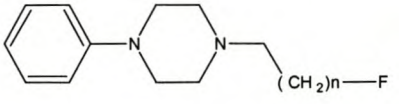

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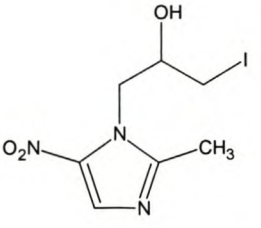

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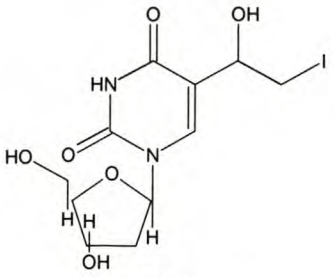

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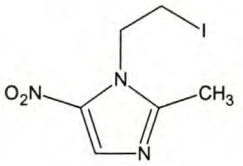

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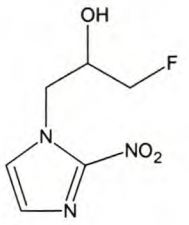
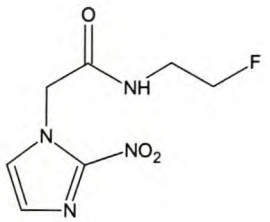


1.16

In another study, fluoromisonidazole (**1.16**), a ^{18}F-labelled compound similar in structure to ornidazole, except for a nitro group replacing the methyl group in the 2-position of the imidazole ring and without any other ring substituents, was claimed to be unstable *in vivo* (*Dolbier et al., 2001*; *Rasey et al., 1999*). Predominantly polar metabolites were identified following renal clearance. These metabolites were not identified, but most probably included free radiofluoride. As it is unlikely that the ring substituents could affect the stability of such a compound, the stability of the ^{131}I-labelled ornidazole must also be questioned. The syntheses of other ^{18}F-labelled nitroimidazole derivatives with improved biological stabilities were also reported. In these compounds the 1-fluoropropan-2-ol group (as in fluoromisonidazole) was replaced with various other prosthetic groups such as an *N*-(2-fluoroethyl)acetamide group (compound **1.17**) (*Rasey et al., 1999*; *Tewson, 1997*), an *N*-(3-fluoropropyl)acetamide group (compound **1.18**) (*Kachur et al., 1999*), and an *N*-(2,2,3,3,3-pentafluoropropyl)acetamide group (compound **1.19**) (*Dolbier (Jr.) et al., 2001*). Other examples of *N*-fluoroalkylated amides are fluoroethyl and fluoropropyl derivatives of spiroperidol (*Shiue et al., 1986*). In the case of the radioiodine-labelled "Flagyl", the iodine is attached to the terminal carbon of an *N*-ethyl unit. The labelled compound was claimed to have a long shelf-life at room temperature with no significant release of free radioiodide (*Tubis et al., 1975*). In the only reference to its stability under physiological conditions it was stated that some radioiodide was metabolically released, but this release was not quantified. The fact that more radiopharmaceuticals containing fluoroalkyl rather than iodoalkyl groups have been documented, can most probably be ascribed to the general higher bond strengths of alkyl fluorides (444 kJ/mole on average) as opposed to alkyl iodides (222 kJ/mole on average) (*Coenen et al., 1983*; *Weast, 1982*).



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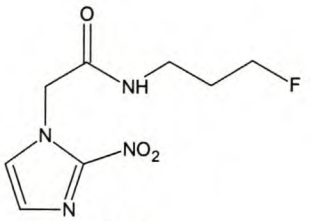

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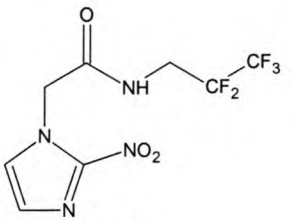
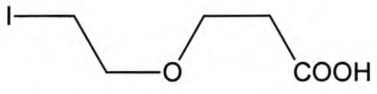


1.19

1.2.3 Radiopharmaceuticals containing the β-Haloethoxyl Moiety.

Another type of prosthetic group that has come to the attention in recent years, and of which the advantages have been described (*Hamant et al., 1994*), is the so-called β-iodoethoxyl moiety. The unusual stability of β-iodoethyl ethers was pointed out more than a century ago (*Baumstark, 1874*; *Demole, 1876*) and was later confirmed by Tasker and Purves (1949). A direct comparison of the radiochemical stabilities of two radioiodine-labelled ω-iodo carboxylic acids showed a remarkable difference in their respective *in vitro* stability in human serum albumin (*Hamant et al., 1994*; *Robinson (Jr.) & Lee, 1975*). 6-Iodohexanoic acid (**1.20**) showed more than 85% deiodination after one hour, while its analogue, in which a methylene group situated in a β-position relative to the radiohalogen had been substituted by an oxygen atom (**1.21**), showed less than 5% deiodination after two days.

1.20



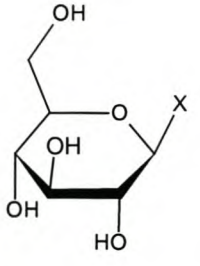
1.21

The radiosynthesis of the fatty acid **1.21**, carried out by isotope exchange in acetone, required heating at a high temperature (105°C). This in itself reflected the stability of this type of C-I bond towards displacement reactions (*Hamant et al., 1994)*, which can be attributed to the β-effect of the oxygen substituent (*Fleet, 1989*). This β-effect can be better understood in the

light of the arguments raised by French *et al.*, (1993). During the development of a series of ^{18}F-labelled estrogen derivatives used for non-invasive diagnostic investigations of breast cancer, several observations regarding metabolic defluorination of some of their compounds were made. The compounds containing fluoroethyl groups showed significantly higher levels of defluorination than those containing fluoroethoxy groups. On the other hand, the estrogens containing fluoropropoxy groups (with the ether oxygen situated gamma with respect to the C-F bond) showed somewhat higher levels of defluorination than those with fluoroethoxy groups. In another publication, French *et al.*, (1991) speculated that the positioning of a heteroatom (oxygen or nitrogen) beta to a carbon-fluorine bond might protect this bond from metabolic cleavage. The metabolism of halogenated alkanes generally involves a preferred hydroxylation at the halogen-bearing carbon, followed by elimination of the halide ion (*Anders & Pohl, 1985*). The metabolic lability of the C-F bond will therefore be determined by the reactivity of the C(F)-H bond towards hydroxylation (*French et al., 1991*). Two mechanisms could play a role in the activating effect of fluorine towards hydroxylation at this site. A resonance mechanism will cause electron donation that will stabilise an incipient radical or carbenium ion intermediate in the hydroxylation process. This mechanism outweighs electron withdrawal by an inductive mechanism, which destabilises such intermediates, and therefore the first mechanism will predominate. However, when a β-heteroatom substituent is present, it will provide an additional metabolically deactivating inductive effect as it is too far removed to increase electron density by resonance (*French et al., 1991*). This will result in a reduction of the reactivity of the beta-situated C-H bonds towards oxidative hydroxylation (*French et al., 1993*). These arguments are most probably also applicable to other halogens such as iodine, and this is indeed confirmed by the ever-increasing number of documented examples of radiopharmaceuticals containing the β-iodoethoxy moiety.

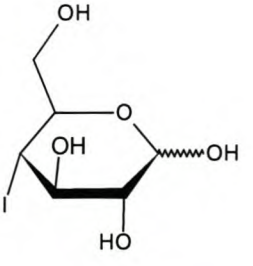
Various examples of radiopharmaceuticals containing this moiety are documented. Besides the labelled fatty acids mentioned above, in which the moiety forms part of a straight aliphatic carbon chain, the syntheses of radiolabelled ω-iodoalkyl glucosides (**1.22** and **1.23**) (*Bignan, et al., 1993*), two D-glucose analogues, namely 4-deoxy-4-iodo-D-glucose (**1.24**) (*Abbadi et al.*,

1997) and 6-deoxy-6-iodo-D-glucose (**1.25**) (*Charronneau et al., 1998*), as well as *N*-substituted radioiodinated nitroimidazole analogues such as iodoazomycin acyclonucleoside (**1.26**) (*Srivastava et al., 1990*; *Srivastava et al., 1991*), and an iodo-β-D-arabinofuranosyl-2-nitroimidazole analogue (**1.27**) (*Mannan et al., 1991*) are also documented. In the compounds **1.22** and **1.23**, the β-iodoethoxyl moiety is not included in the glucoside ring, but forms part of the aliphatic substituent X at *C*-1. In the glucose analogue **1.24**, the iodine label is attached directly to the ring at a secondary carbon, having replaced the OH-group at the *C*-4 position, while the glucoside ring oxygen atom serves as the β-oxygen atom. In the second glucose analogue, **1.25**, the iodine is attached to an exocyclic methylene group, but is also in a β-position relative to the ring oxygen. In the imidazole derivative **1.26**, the β-iodoethoxyl unit is linked to the *N*-1 nitrogen via a methylene bridge, while in **1.27** it forms part of the arabinofuranosyl ring that is linked to the *N*-1 nitrogen. In the ^{125}I-labelling of the 5-(1-hydroxy-2-iodoethyl)-2'-deoxyuridine (**1.14**) described earlier, a 1-methoxy derivative (**1.28**) was also labelled (*Iwashina et al., 1990*). The 1-methoxy-2-iodoethyl unit in this derivative can also be classified as a β-iodoethyl ether moiety.



X = OCH_2CH_2I **1.22**

X = $OCH_2CH_2OCH_2CH_2I$ **1.23**



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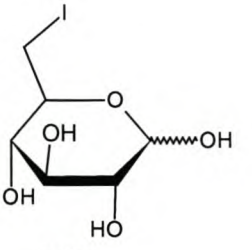

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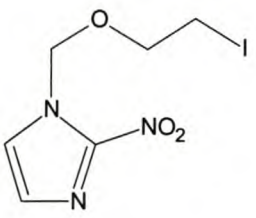

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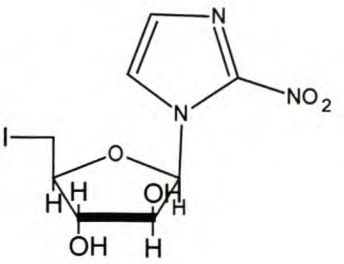
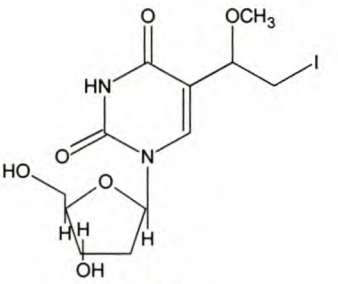
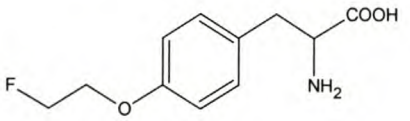


1.27



1.28

In addition to these radioiodinated compounds, some radiofluorinated compounds containing this moiety are also documented. In the ^{18}F-labelled tyrosine derivative [*O*-(2-fluoroethyl)-L-tyrosine (**1.29**)], for example, the tyrosine hydroxyl group is derivatised by fluoroalkylation (*Wester et al., 1999*). In this compound, the hydroxyl oxygen atom serves as the β-oxygen. [^{18}F]Fluoroethoxy-benzovesamicol (**1.30**), a radiotracer for cholinergic neurons, is another example of this type of substitution. This type of [^{18}F]fluoroethyl ether was first reported by French *et al.*, (1993). No examples of similar radioiodo-ethoxy compounds could be found in literature, presumably because the facile incorporation of an iodine atom into a phenolic ring by means of electrophilic substitution is a more attractive alternative, especially when the ring is activated by an electron-donating hydroxyl group, in contrast to electrophilic substitution with fluorine which results in low yields (*Wester et al., 1999*). Finally, another interesting example in which radiofluorine is incorporated into a β-position relative to an aliphatic oxygen atom is shown in compound **1.32**. A 2-nitroimidazole nucleoside analog, 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (**1.31**) was converted into its 2-[^{18}F]fluoro analog (**1.32**) by firstly protecting one of the two equivalent hydroxyl groups by acetylation, converting the other one to a tosylate, followed by substitution with ^{18}F and subsequent deprotection (*Wada et al., 2000*). This radiopharmaceutical was developed as an alternative to the previously reported [^{18}F]fluoromisonidazole (**1.16**) in order to improve its biological properties. No mention, however, was made of the *in vivo* stability of this new radiopharmaceutical. The application of this 2-[^{18}F]fluoro analog for imaging ischemic but viable myocardium was documented recently (*Kaneta et al., 2002*).



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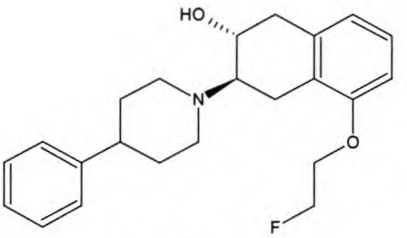

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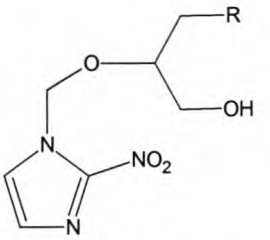


R = OH **1.31**
R = F **1.32**

1.2.4 Concluding Remarks on Prosthetic Groups

The use of prosthetic groups in radiopharmaceutical chemistry has become an increasingly important tool for the radiochemist. Their most important feature should be to impart optimum radiochemical stability to a radiopharmaceutical without significantly affecting its biological properties. Small aliphatic units such as iodoallyl groups would be mostly preferred, especially when designing radiopharmaceuticals with relatively low molecular masses. Additional features such as the high stability of vinyl iodides and the facile labelling of their precursors make iodoallyl groups particularly attractive. It is possible, however, that certain structural requirements with regard to a target compound could restrict or rule out the use of these groups. There will therefore be a need for alternative prosthetic groups that might not necessarily possess the advantageous features of the iodovinyl unit, but could structurally be more reconcilable with the parent compound. The β-iodoethoxyl moiety offers the radiochemist such an alternative option. Many target molecules, such as glucose derivatives, already contain an aliphatic oxygen atom in their structures, which makes the use of a β-iodoethoxyl prosthetic group very attractive for radiolabelling purposes. Since this type of prosthetic group has been utilised relatively infrequently, little information is available, especially, on its radiochemical stability. Some contradictory statements have also been made about the *in vivo* stability of a radioiodinated nitroimidazole derivative containing such a moiety. According to Srivastava *et al.*, (1990), the injected radiolabelled compound iodoazomycin acyclonucleoside (**1.26**) resulted in low *in vivo* deiodination in mice, whereas Hasan *et al.*, (1991) claimed that the same compound resulted in high levels of radioactivity in the blood, spleen and lungs, probably due to *in vivo* deiodination. This could also be as a result

of the compound having been metabolised (*Srivastava et al., 1991*). Apparently more research is needed in order to determine the stability of a radiolabelled β-iodoethoxyl moiety by comparing it with the so-called "gold standards" such as the iodovinyl unit (which forms part of the iodoallyl group), as well as to find methods to further stabilise the moiety.

1.3 OBJECTIVES OF THIS STUDY

The primary objective of this research project was to investigate the utilisation of radioiodinated prosthetic groups in radiopharmaceutical chemistry with particular regard to their radiochemical stabilities under semi-physiological conditions. The emphasis was placed on the β-iodoethoxyl moiety. The synthesis of compounds containing other moieties such as the iodovinyl unit was only done for reference purposes. The aim was to compare the stabilities of the β-iodoethoxyl compounds with these iodovinyl compounds, which are referred to as the "gold standards". In order to avoid using these small units on their own because of their unacceptable physical properties such as their high volatilities, it was decided to couple them to various carrier or model compounds before radioiodination. The following tasks were therefore undertaken: (As a rule-of-thumb, the term "β-iodoethoxyl" refers to the position of the iodine atom (β) relative to the ether oxygen atom, while the term "β-oxygen" refers to the position of the oxygen atom (β) relative to the iodine atom).

(i) In a pilot study, a systematic comparative investigation of the *in vitro* radiochemical stabilities of model compounds containing radioiodinated β-iodoethoxyl units and derivatives thereof, as well as those of control references lacking a β-oxygen atom, was undertaken. These target derivatives were designed to investigate how various neighbouring alkyl and aryl groups situated in various positions relative to the β-oxygen, would affect the radiochemical stability of the radioiodinated compounds. At this stage no *deliberate* attempts were made to improve the stability of the prosthetic groups by altering the structures of these moieties. The aim was to investigate stability trends. For the purpose of this study, aromatic ethers or ethers containing an aromatic group were

selected as model compounds to avoid complications arising from the high volatility of aliphatic ethers. All of this was done on phenolic or benzyloxy compounds in which the phenolic or benzyloxy oxygen served as the β-oxygen atom.

(ii) Based on the results obtained in the pilot study, a few β-iodoalkoxyl moieties with certain structural features, as well as the unmodified β-iodoethoxyl moiety, were selected for coupling to various carrier molecules[3], hereafter called carriers. In addition to the moieties emerging from the pilot study, another slightly different unit, the iodo-analogue of the 1-[2-fluoro-1-(hydroxymethyl)-ethoxy]methyl moiety present in 1-[(1-fluoro-3-hydroxyprop-2-yloxy)methyl]-2-nitroimidazole **1.32**, was also selected. It was decided to attach it only to a heterocyclic amine as explained in objective (iii). Finally, two units lacking a β-oxygen were also selected. The reason for their selection is explained in Section 2.3, but they were supposed to serve a similar function as the control references used in the pilot study.

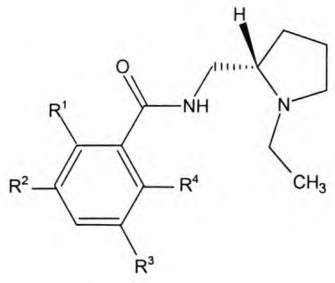
(iii) Three carriers, belonging to two different types of compounds, namely a benzamide containing a phenolic group, and two heterocyclic amines, were selected in order to determine the selected moieties' behaviour in different chemical environments. With the exception of one of the heterocyclic amines, namely benzotriazole, these carriers were not model compounds, in contrast to the compounds used in the pilot study, but had the chemical features of established radiopharmaceutical compounds. The rationale behind this was that, if the prosthetic moieties linked to these compounds showed appreciable levels of stability, their radioactively labelled analogues could be considered to be radiopharmaceuticals.

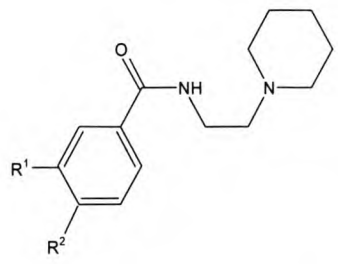
A benzamide containing a phenolic hydroxyl group was selected as the first type of carrier. The phenolic group present in these molecules was also present in some of the selected model compounds. Such a structure was chosen because radioiodinated

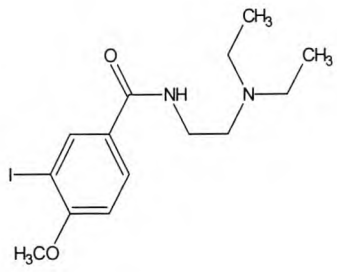
benzamides are widely used as radiotracers. Benzamides furthermore are easy to prepare. (*S*)-(-)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxybenzamide (IBZM) (**1.33**) (*Kung & Kung, 1989*; *Kung et al., 1991*; *Bobeldijk et al., 1990*), 2,3-dimethoxy-(*S*)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodobenzamide (Epidepride) (**1.34**) (*Clanton et al., 1991*; *Mulligan et al., 1999*), 4-iodo-*N*-(2-piperidin-1-ylethyl)benzamide (IPAB) (**1.35**) (*John et al., 1993*), 3-iodo-4-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide (PIMBA) (**1.36**) (*John et al., 1999*) and *N*-(2-diethylaminoethyl)-3-iodo-4-methoxybenzamide (IMBA) (**1.37**) (*Nicholl et al., 1997*) are but a few of the radioiodinated benzamides that have been reported in the literature. Radioiodinated benzamides have an affinity for melanocytes (*Mohammed et al., 1997*) and can therefore be used for radioimaging of various types of tumours such as melanoma (*John et al., 1993*; *Nicholl et al., 1997*; *Mohammed et al., 1997*) and breast cancer (*John et al., 1999*). Compounds such as IBZM display significant antidopaminergic activity (*Hogberg et al., 1987*) and are used for dopamine D-2 receptor imaging in the central nervous system. Many structure-activity relationship studies have been carried out on benzamides in order to establish optimum biological characteristics. Of these, structural variations on the phenyl ring, such as introduction of a methoxy group, resulted in considerable improvements in their biological characteristics, for instance high melanoma affinity and excellent melanoma/non-target tissue ratios (*Mohammed et al., 1997*). A few [^{18}F]fluoroalkyl- as well as [^{18}F]fluoroalkoxy substituted benzamides (compounds **1.38** to **1.40**) have been documented (*De Paulis et al., 1991*); however, no iodinated analogues of a similar kind could be found. In all the documented examples of radioiodinated benzamides, the radioiodine atom is directly attached to the aromatic ring as shown in compounds **1.33** to **1.37**. In this study, however, it was decided to synthesise radioiodinated benzamides with the iodine attached to an aliphatic carbon, as in the fluorinated analogues **1.39** and **1.40**, with the phenolic oxygen serving as the stabilising β-heteroatom. The synthetic strategy was the *O*-alkylation of the free phenolic hydroxyl group of a pre-synthesised benzamide derivative with the appropriate prosthetic group

[3] The term carrier molecule or carrier refers to the parent molecule to which the moiety is coupled

containing suitable active sites for the condensation as well as iodination steps. While the primary purpose of this exercise was to investigate the radiochemical stabilities of these compounds and not necessarily to improve on existing benzamide radiotracers, the stability results were expected to indicate whether there might be any advantages in pursuing this method of benzamide labelling.







$R^1 = OH$, $R^2 = I$, $R^3 = H$, $R^4 = OCH_3$ **1.33**
$R^1 = H$, $R^2 = I$, $R^3 = R^4 = OCH_3$ **1.34**
$R^1 = H$, $R^2 = (CH_2)_3F$, $R^3 = R^4 = OCH_3$ **1.38**
$R^1 = H$, $R^2 = Br$, $R^3 = O(CH_2)_2F$, $R^4 = OCH_3$ **1.39**
$R^1 = H$, $R^2 = Br$, $R^3 = OCH_3$, $R^4 = O(CH_2)_2F$ **1.40**

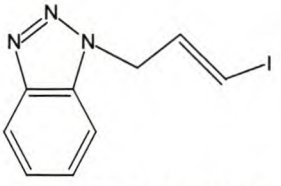
$R^1 = H$, $R^2 = I$ **1.35**
$R^1 = I$, $R^2 = OCH_3$ **1.36**

1.37

A heterocyclic aromatic amine with a ring nitrogen atom for *N*-alkylation was selected as the second type of carrier. Although no information regarding the use of benzotriazoles as radiopharmaceuticals could be found in the literature, benzotriazole was chosen to serve as a model compound. The objective was to attach a few of the prosthetic moieties, similar to those used in the pilot study and in the benzamide compounds, to the nitrogen atom of benzotriazole in order to establish whether similar stability trends would be observed when the prosthetic groups are present in different chemical environments. 2-Methyl-5-nitroimidazole was also selected as a heterocyclic amine because of the widespread utilisation of nitroimidazoles in radiopharmaceutical chemistry as discussed in Sections 1.2.2 and 1.2.3. The infection-seeking compound iodo-ornidazole **1.13** also has a 2-methyl-5-nitroimidazole heterocyclic ring in its structure. In addition to its condensation

with a few of the stabilised moieties identified in the pilot study, it was also decided to condense the nitroimidazole with the 1-[2-halo-1-(hydroxymethyl)-ethoxy]methyl moiety as discussed in objective (ii). In the light of the questionable stability of radioiodinated ornidazole (as mentioned in Section 1.2.2), this study might result in the development of an alternative prosthetic group with improved stability. This could create an opportunity to develop improved radiopharmaceuticals with structures closely related to those of existing ones. Similar synthetic methods were used in the preparation of some of the *N*-alkylated prosthetic moieties, using the two different heterocyclic amines as starting materials.

(iv) In addition to the synthesis of the *O*- and *N*-alkylated β-iodoalkoxyl compounds, their respective iodoallyl analogues were also synthesised for reference purposes. The synthesis of 1-(3-[^{123}I]iodo-2-propen-1-yl)benzotriazole was carried out in a separate study (*Visser, 2002*), but the results regarding its radiochemical stability will be presented and discussed in this work.



1-(3-iodo-2-propen-1-yl)benzotriazole

(v) The optimisation of reaction conditions in organic synthesis with the aim to improve product yields was not an objective of this study, neither was the investigation of various labelling techniques of the prosthetic groups with the aim to optimise radiochemical yields. With the exception of the comparative radioiodinations of two different nitroimidazole precursors (see Section 3.4.5.3), the kinetics of the labelling reactions were not studied.

(vi) In this study, only *in vitro* stability tests in human blood serum were carried out on the various radioiodinated compounds. This technique has already been used by other

workers (*Harapanhalli et al., 1998*; *Robinson (Jr.) & Lee, 1975*) to make an approximate estimate of the radiochemical stability of a radiolabelled compound. The testing of the *in vitro* serum stability of a chemical does not necessarily reflect its *in vivo* characteristics (*Harapanhalli et al., 1998*). The various biological processes such as metabolism and catabolism that occur in the body can also result in deiodination (*Coenen et al., 1983*). It was, however, not intended to develop radiopharmaceuticals that would necessarily be biologically stable, but to observe stability trends. This method of stability evaluation does not produce quantifiable, but only relative results, which were expected to give a fair indication of the expected *in vivo* stability trends.

To summarise, this study comprised three components, namely: (a) organic synthetic chemistry (b) radiochemistry (radiolabelling) and (c) stability evaluation. The first objective was to synthesise precursor molecules suitable for labelling with radioiodine. These precursors consisted of a suitable carrier molecule (parent molecule) covalently bonded to the appropriate prosthetic group, which in turn had a leaving group in an appropriate position that could be substituted with iodine. The leaving group mostly used in this work was the *p*-toluenesulphonate (tosylate) group. It was anticipated that an iodide-for-tosylate substitution reaction would occur more readily than, for instance, halogen exchange in a Finkelstein substitution reaction. For this reason, a few bromo compounds were rather converted to hydroxy compounds, followed by tosylation, instead of directly iodinating the bromo compounds. In some cases the more reactive trifluoromethanesulphonate (triflate) group was also used as a leaving group, while one of the reference compounds (a vinyl derivative) was equipped with a tri-*n*-butylstannyl group. The second objective was to convert the precursors to radioiodinated compounds by introducing the radioiodine atom regioselectively at the position of the leaving group. Finally, the labelled compounds were incubated in blood serum at 37°C to determine their *in vitro* radiochemical stability. The extent of release of free radioiodine was used as an indicator for stability assessment.

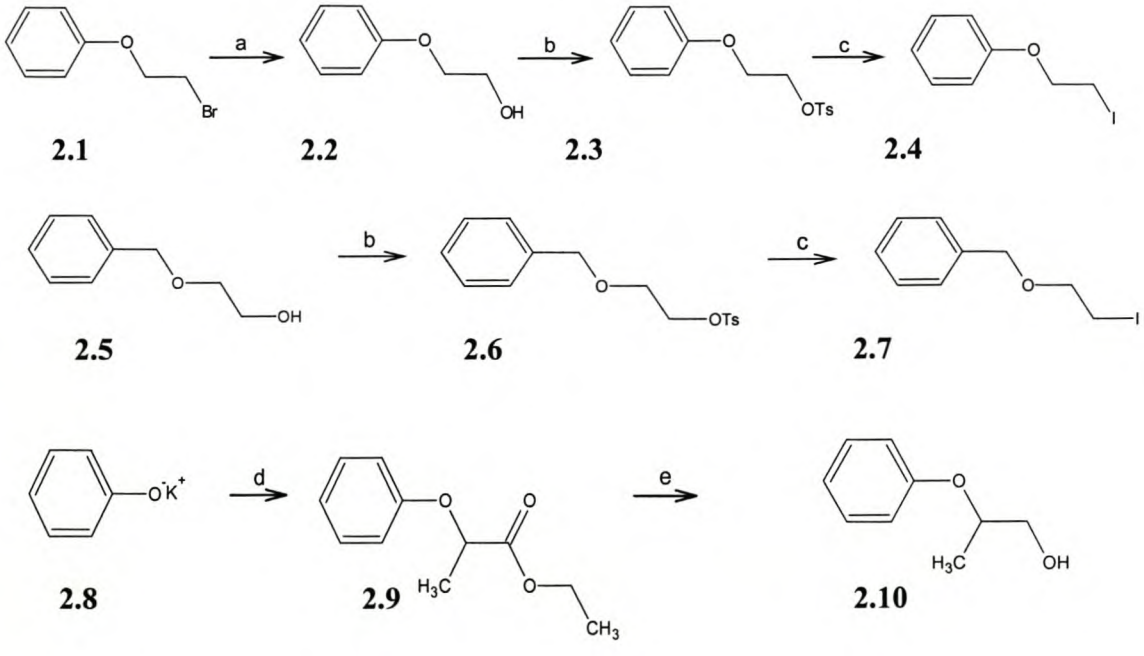
CHAPTER 2

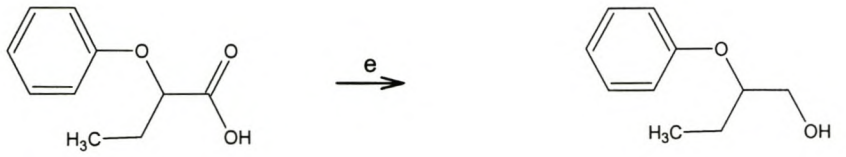
SYNTHESIS OF LABELLING PRECURSORS AND NON-RADIOACTIVE IODO DERIVATIVES

2.1 PILOT STUDY

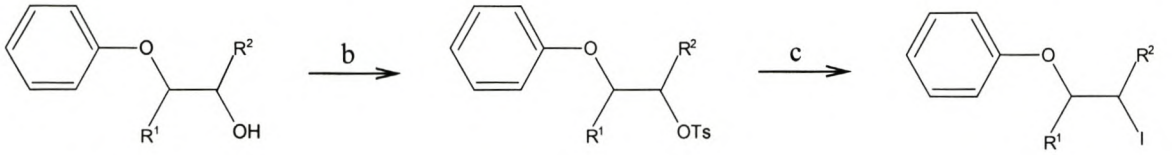
Simple model compounds were selected for this preliminary investigation of the stability of β-iodoalkylethers. The model compounds were all β-hydroxy ethers, a few of which were commercially available. They were composed of either phenolic or benzyloxy groups attached to aliphatic chains containing the free hydroxyl group. The β-hydroxy ethers were converted to the required precursors by tosylation of the free hydroxyl group. The tosylates, prepared according to the method of Argentini *et al.* (1981), were converted to the corresponding iodo derivatives by heating them with sodium iodide in acetone as solvent. These iodinated compounds only served as reference materials for characterisation and identification of the radioiodinated species. The synthesis of the model compounds that were not commercially available, the precursors, and the iodinated compounds was carried out according to the reaction schemes shown in Scheme 2.1. The β-hydroxy ether **2.2** was synthesised by hydrolysis of β-bromophenetole **2.1** in a mixture of H_2O/HMPT according to the method of Hutchins and Taffer (1983). Subsequent tosylation of the hydroxy group, followed by iodination of the tosylate, gave 2-phenoxyethyl *p*-toluenesulphonate **2.3** and 1-iodo-2-phenoxyethane **2.4** respectively. Commercially available 2-benzyloxyethanol **2.5** was likewise converted into 2-benzyloxyethyl *p*-toluenesulphonate **2.6** and 1-benzyloxy-2-iodoethane **2.7** respectively. 2-Phenoxy-1-propanol **2.10** was obtained by the condensation of potassium phenoxide **2.8** with ethyl 2-bromopropionate in the presence of a crown ether catalyst, according to the method of Rall *et al.* (1976), followed by reduction of the resulting ethyl 2-phenoxypropionate **2.9** with lithiumaluminiumhydride in THF. Tosylation of **2.10** resulted in 2-phenoxyprop-1-yl *p*-toluenesulphonate **2.11**. Iodination of **2.11** gave 1-iodo-2-phenoxypropane **2.12**. Similar

treatment of commercially available 1-phenoxypropan-2-ol **2.13** gave 1-phenoxyprop-2-yl *p*-toluenesulphonate **2.14** and 2-iodo-1-phenoxypropane **2.15** respectively. 2-Phenoxy-1-butanol **2.17** was obtained by reduction of 2-phenoxybutyric acid **2.16**. Its respective tosylated and iodinated analogues **2.18** and **2.19** were prepared as before. The same method was used for the preparation of 2-methoxy-2-phenylethyl *p*-toluenesulphonate **2.21** and its iodinated analogue **2.22** from commercially available 2-methoxy-2-phenylethanol **2.20**. In addition to these β-iodoalkoxy compounds, two reference compounds, one containing an ether oxygen in a *gamma* position relative to the iodine (1-iodo-3-phenoxypropane **2.26**), and the other without an ether oxygen (1-iodo-2-phenylethane **2.30**), were also prepared. Compound **2.26** was prepared by hydrolysis of 1-bromo-3-phenoxypropane **2.23** in aqueous HMPT, followed by tosylation of the formed 3-phenoxy-1-propanol **2.24** and subsequent iodide-for-tosylate exchange of 3-phenoxyprop-1-yl *p*-toluenesulphonate **2.25**. Compound **2.30** was prepared in a similar fashion, starting from 1-bromo-2-phenylethane **2.27**, and subsequent tosylation and iodination of 2-phenylethyl alcohol **2.28** and 2-phenylethyl *p*-toluenesulphonate **2.29**, respectively.

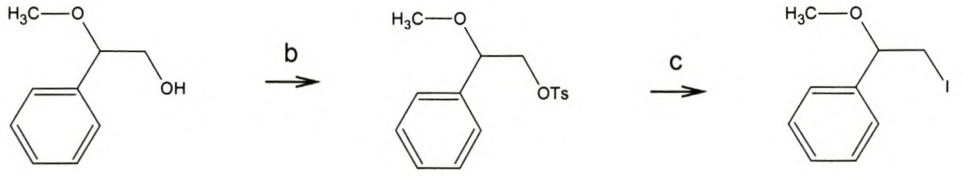




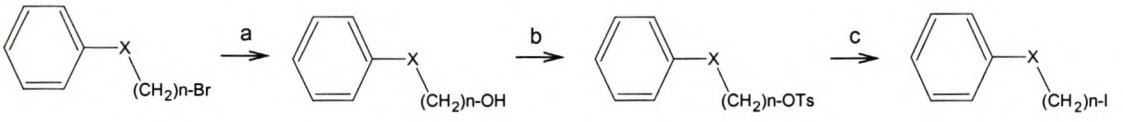
2.16 **2.17**



2.10 $R^1 = CH_3$; $R^2 = H$ **2.11** $R^1 = CH_3$; $R^2 = H$ **2.12** $R^1 = CH_3$; $R^2 = H$
2.13 $R^1 = H$; $R^2 = CH_3$ **2.14** $R^1 = H$; $R^2 = CH_3$ **2.15** $R^1 = H$; $R^2 = CH_3$
2.17 $R^1 = C_2H_5$; $R^2 = H$ **2.18** $R^1 = C_2H_5$; $R^2 = H$ **2.19** $R^1 = C_2H_5$; $R^2 = H$



2.20 **2.21** **2.22**



x = O; n = 3 **2.23** x = O; n = 3 **2.24** x = O; n = 3 **2.25** x = O; n = 3 **2.26**
x = CH_2; n = 1 **2.27** x = CH_2; n = 1 **2.28** x = CH_2; n = 1 **2.29** x = CH_2; n = 1 **2.30**

a. 15 % H_2O/HMPT; 130°C
b. *p*-TsCl / pyridine; 5°C
c. NaI/dioxane/acetone; heat under reflux
d. (i) 18-crown-6 / CH_3CN (ii) ethyl 2-bromopropionate
e. $LiAlH_4$/THF /ether; reflux

Scheme 2.1 Synthesis of the model alcohols, followed by tosylation and iodination to yield β-iodoalkylether derivatives and reference compounds.

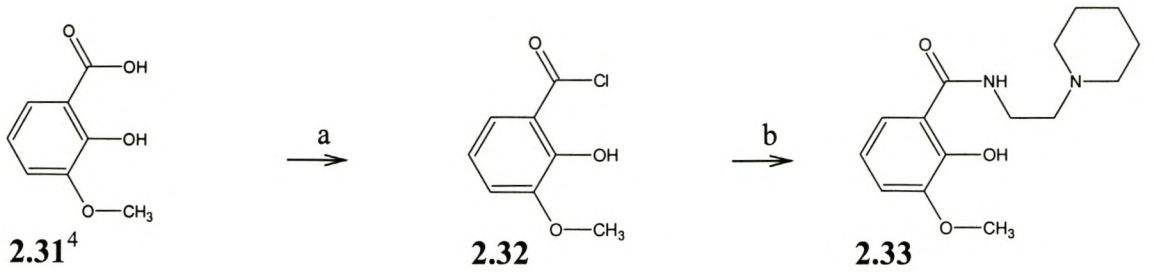
As mentioned in the statement of objectives, a few of these β-iodoalkoxyl moieties that showed enhanced stability, together with their non-stabilised parent moieties, were selected as prosthetic moieties for coupling to the selected carriers. It is important to mention that methods used to couple them to the simple model compounds would not necessarily be suitable for their coupling to the more complex carriers. The presence of sensitive groups (amides) in the latter type of compounds could be vulnerable to the synthetic conditions required for these transformations. The challenge was therefore to design suitable synthetic procedures.

The stabilised moieties selected were of the type displayed in 1-iodo-2-phenoxypropane **2.12** and 2-iodo-1-phenoxy-propane **2.15**. As mentioned before, two different types of carriers were used. The respective amines were readily available commercially, but the benzamide had to be synthesised, using conventional methods. No efforts were made to optimise the yields of the intermediates or the final products, but where necessary they were purified to the highest level possible, especially for characterisation purposes.

2.2 SYNTHESIS OF THE BENZAMIDE DERIVATIVES

The analytical chromatographic methods that are been referred to from here onwards are described in Section 6.2.

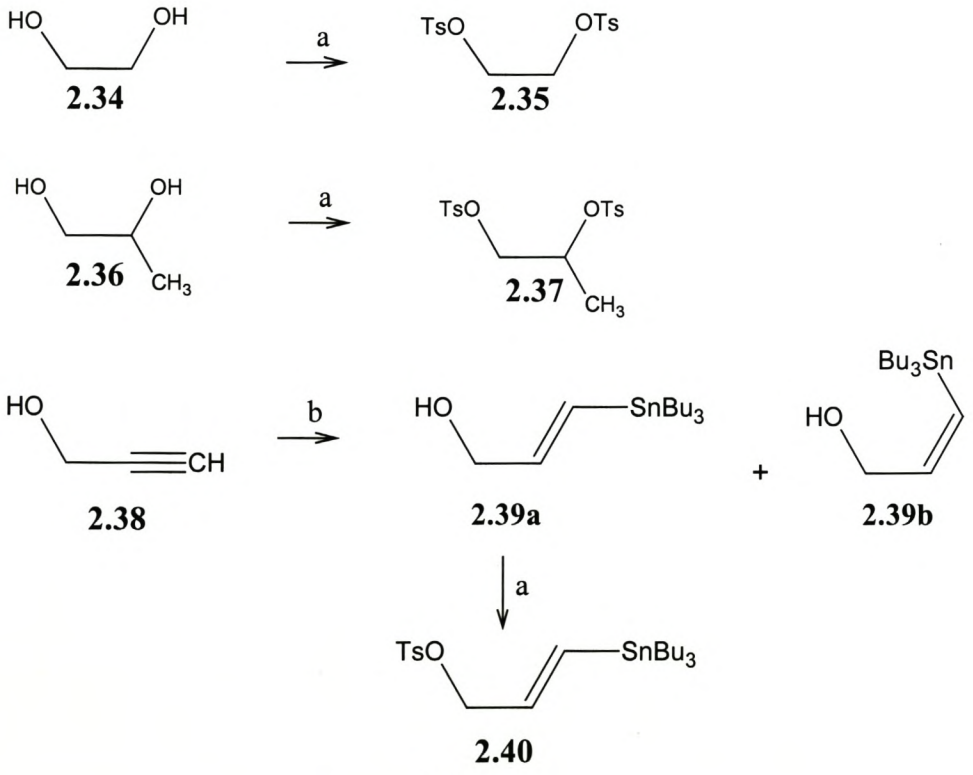
In this section the synthetic procedures for the various benzamide derivatives containing the pre-selected prosthetic moieties are discussed. The synthetic routes leading to the benzamide carrier **2.33**, all the desired reagents, precursors and iodinated compounds are summarised in Schemes 2.2 to 2.4, and are followed by a detailed discussion of the synthetic procedures.



2.31[4] **2.32** **2.33**

a. Thionyl chloride
b. 1-(2-aminoethyl)piperidine

Scheme 2.2 Synthesis of the benzamide carrier **2.33**

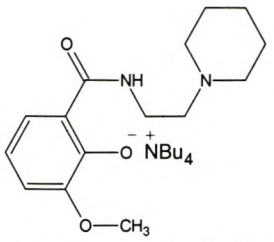


2.34 **2.35** **2.36** **2.37** **2.38** **2.39a** **2.39b** **2.40**

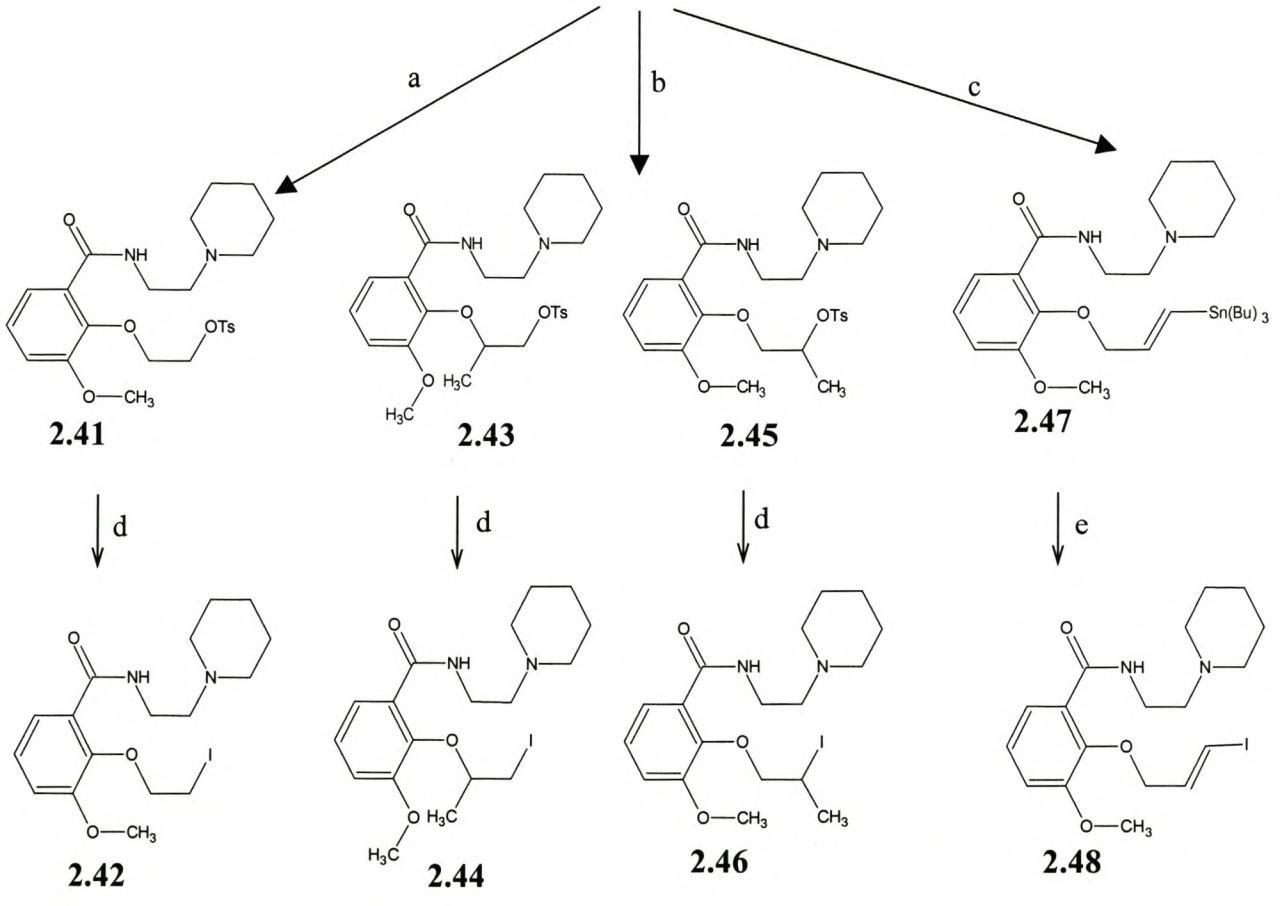
a. *p*-TsCl
b. Bu_3SnH

Scheme 2.3 Synthesis of the alkylating agents for *O*-alkylation of the benzamide carrier

[4] The drawing programme automatically terminates an aliphatic chain with a methyl group



2.33 tetrabutylammonium phenoxide



a. Ethylene di-*p*-toluenesulphonate **2.35**
b. Propylene 1,2-di-*p*-toluenesulphonate **2.37**
c. (*E*)-3-(Tributylstannyl)prop-2-en-1-yl *p*-toluenesulphonate **2.40**
d. NaI
e. Iodine

Scheme 2.4 Synthesis of the benzamide precursors and iodinated compounds

The type of benzamide carrier was selected arbitrarily. The main criterion was that it should contain a free hydroxyl group somewhere on the aromatic ring. It was also decided to have a methoxy group on the ring, as many established radiopharmaceuticals of this class contain this group. The relative position of the OH group with regard to the amide side chain was regarded as irrelevant as it was unlikely that this would affect the stability of the prosthetic groups. The starting material also had to contain a carboxylic group that could be converted to an amide group. 3-Methoxysalicylic acid **2.31** was chosen as the starting material.

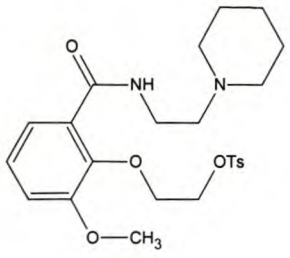
2.2.1 Synthesis of Carrier **2.33**

The reaction sequence is illustrated in Scheme 2.2, which is represented below. The synthesis was based on the method proposed by John *et al.* (1993). The carboxylic function of 3-methoxysalicylic acid **2.31** was converted to the acid chloride by means of thionyl chloride, affording the acyl chloride intermediate **2.32**. Subsequent condensation of **2.32** with 1-(2-aminoethyl)piperidine gave 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33**. In a similar sequence, Kung *et al.* (1988b) converted 3-iodo-6-methoxysalicylic acid via the acid chloride to a benzamide without protecting the phenolic hydroxyl group. This group was therefore also not protected in this synthesis.

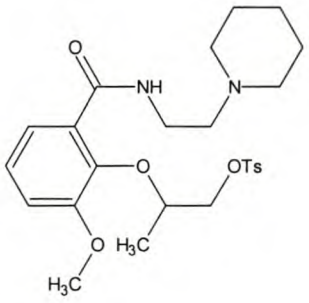
Some by-products were formed during the synthesis, presumably due to reactions with the phenolic hydroxyl group. Silica gel column chromatography gave a moderate yield of approximately 38%, which was sufficient for the subsequent steps.

2.2.2 Synthesis of the Benzamide Precursors

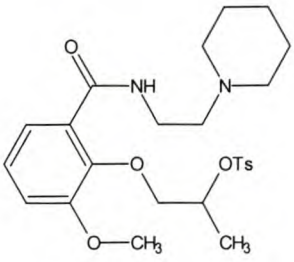
The term "benzamide precursors" refers to the iodination precursors containing a benzamide group. On the basis of the previously mentioned considerations, the benzamide derivatives mentioned below were selected as precursors for radioiodination:



2.41



2.43

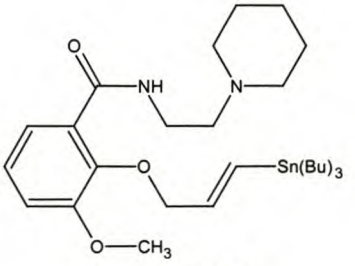


2.45

The strategy was to *O*-alkylate the phenolic hydroxyl group of the carrier 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33** with various alkylating agents to produce precursors, each having a good leaving group in the β-position. Nucleophilic substitution of this leaving group with iodine was expected to result in the regioselective incorporation of an iodine atom in a β-position relative to the phenolic oxygen atom. Various reaction routes and alkylating agents were considered. In the pilot study, 1-iodo-2-phenoxypropane **2.12** was synthesised by condensing potassium phenoxide with the ethyl ester of 2-bromopropionic acid under phase transfer conditions, followed by reduction of the ester **2.9** to the corresponding alcohol **2.10**, subsequent tosylation to give **2.11** and substitution with iodide to give **2.12**. Such a strategy would probably not be feasible for the transformation of benzamide carrier **2.33** to the various precursors due to the relatively harsh reaction conditions that have to be used. Conversion to the potassium salt could probably result in hydrolysis of the amide, while the reduction step could also result in reduction of the amide. One viable option appeared to be the alkylation of the OH group with bifunctional agents such as 2-bromo-1-ethanol, 2-bromo-1-propanol or 1-bromo-2-propanol, followed by conversion of the free hydroxyl group to a tosylate group, to give precursors **2.41**, **2.43** and **2.45** respectively. Another, shorter, route would be to make use of ditosylates such as ethylene di-*p*-toluenesulphonate **2.35** or propylene 1,2-di-*p*-toluenesulphonate **2.37** as alkylating reagents. According to the method proposed by Mulholland *et al.* (1993), the phenolic hydroxyl group of (-)-(2R,3R)-trans-2-hydroxy-3-(4-phenylpiperidino)-5-hydroxytetralin was alkylated with an excess of ethylene di-*p*-toluenesulphonate after formation of the phenoxide with tetrabutylammonium hydroxide. Being a symmetrical ditosylate, this reagent could be expected to yield only one condensation

product, namely (-)-(2R,3R)-trans-2-hydroxy-3-(4-phenylpiperidino)-5-[2-(*p*-toluenesulphonyloxy)ethoxy]tetralin, while condensation of the formed product with another molecule of the phenoxide would be countered by the large excess of ditosylate used. The advantage of this method was that it is essentially a one-step reaction, without the need for a tosylation step. A possible disadvantage was that, when using un-symmetrical ditosylates such as propylene 1,2-di-*p*-toluenesulphonate, inseparable isomeric mixtures could be formed. Nevertheless, it was decided to investigate this method because of its anticipated simplicity.

In addition to these β-tosyloxyalkoxyl precursors, a precursor for the synthesis of an iodoallyl compound was also needed to serve as a reference, as explained in the objectives of this thesis. This precursor, 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[3-(tributylstannyl)prop-2-en-1-yloxy]-benzamide **2.47**, was prepared according to the method of Wang *et al.* (1995). The tributylstannyl group served as the leaving group for displacement with electrophilic iodine. It is important to note that, after such a displacement, the iodine atom would be situated in a gamma position relative to the phenolic oxygen atom and would therefore be stabilised, not by the β-effect of an ether oxygen atom, but by the vinyl group. As in the previous cases, the strategy for the synthesis of compound **2.47** was based on *O*-alkylation of the phenolic hydroxyl group of 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33** with a suitable bifunctional vinylstannane moiety. The prosthetic group (*E*)-3-(tributylstannyl)prop-2-en-1-yl *p*-toluenesulphonate (TBS-PROP-TOS) **2.40**, employed by Musachio and Lever (1992) for the preparation of, amongst others, iodoallyl analogues of spiperone, was used for this purpose.



2.47

2.2.2.1 Synthesis of the precursor 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)ethoxy]benzamide **2.41**

Ethylene di-*p*-toluenesulphonate (TET) **2.35** was easily prepared from ethylene glycol **2.34** where a two-molar excess of *p*-toluenesulphonyl chloride (*p*-TsCl) was used, as shown in Scheme 2.3. The mixture was refluxed in dichloromethane in the presence of triethylamine (TEA), after which the formed TEA.HCl was filtered off and the filtrate concentrated and chromatographed over silica gel. The coupling of TET to the benzamide is shown in Scheme 2.4. Tetrabutylammoniumhydroxide (TBAH) was used to form the phenoxide of 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33**, and this was subsequently refluxed with a nine- to ten-fold molar excess of purified TET in acetonitrile, according to the method of Mulholland *et al.* (1993). Thin-layer chromatography (TLC) (Method 4) indicated total consumption of **2.33** after 1.5 hours, after which the reaction mixture was evaporated to dryness, the residue re-dissolved in chloroform, washed with water, and again evaporated to dryness. The residue was re-dissolved in acetonitrile, excess TET was partially removed by crystallisation at -10°C, and the concentrated mother liquor was chromatographed twice over silica gel to give the purified product in a yield of approximately 57%.

2.2.2.2 Synthesis of the precursors 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[1-(*p*-toluenesulphonyloxy)prop-2-yloxy]benzamide **2.43** and 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)prop-1-yloxy]benzamide **2.45**

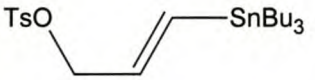
In order to synthesise these two isomeric compounds, it was decided to follow the same synthetic route that was used for the synthesis of precursor **2.41**. The unsymmetrical propylene 1,2-di-*p*-toluenesulphonate (TPT) **2.37** was selected as the alkylating agent. It was expected that if the two isomers were formed simultaneously, they might be separable by chromatography over silica gel. As no example of a similar condensation reaction could be found in the literature, no information was available on the expected ratio in which the isomers would be formed.

TPT was prepared in a similar way as TET, as shown in Scheme 2.3, using propane-1,2-diol **2.36** as the starting material. The reaction mixture was refluxed at an oil bath temperature ranging between 80° and 120°C. Condensation with the phenoxide of the benzamide **2.33**, using a nine- to ten-fold molar excess of purified TPT, was carried out as before (Scheme 2.4). TLC indicated completion of the reaction after 20-30 minutes, after which the reaction mixture was evaporated to dryness. As the removal of excess TPT by crystallisation was unsuccessful, the whole mixture was chromatographed to remove all the excess TPT. This gave the target product containing some residual polar impurities. TLC (Method 5) showed a partial resolution of what was expected to be two isomeric products. Analytical HPLC (Method 1) gave acceptable resolution of the two expected isomers, thereby enabling their relative quantification. The ratio between the more polar compound and the less polar one was approximately 37:63. The two isomers were subsequently separated by column chromatography over silica gel, using diisopropylether/isopropanol/ammonia (85:15:0.5) as mobile phase. A large fraction consisting of a mixture of the two compounds was obtained since a complete separation could not be achieved. Only the fractions highly enriched in a specific isomer were combined separately, resulting in a recovery of approximately 19% of the less polar compound and 14% of the more polar one. The correct structure assignments of the two compounds were made possible by means of proton magnetic resonance spectroscopy (^{1}H NMR). The methine proton adjacent to the phenoxide in compound **2.43** was expected to resonate slightly further upfield (lower δ) than the one adjacent to the tosylate group in compound **2.45**. The spectrum of the less polar compound had a lower chemical shift (δ 4.70-4.73) for the methine proton than that of the more polar one (δ 4.98-5.03). This difference was quite subtle due to the small difference in chemical shifts caused by the tosylate and phenoxide functional groups, respectively. More evidence was obtained from the ^{1}H NMR spectra of the iodinated analogues prepared from the respective tosylates. The less polar tosylate yielded an iodinated compound of which the ^{1}H NMR spectrum showed a pair of methylene protons resonating at relatively higher field (δ 3.44-3.51). The more polar tosylate, on the other hand, gave an iodinated compound of which the ^{1}H NMR spectrum showed a pair of methylene proton signals at lower field (δ 4.12-4.26). Due to the shielding effect exerted by an iodine

atom, it would be expected that the methylene protons resonating at δ 3.44-3.51 would be geminal to an iodine atom (such as in structure **2.44**), rather than an oxygen atom, while those at lower field would be geminal to an oxygen atom (such as in structure **2.46**). The tosylate structure **2.43** was therefore assigned to the less polar tosylate, which gave the iodo compound **2.44**, and **2.45** to the more polar tosylate, which gave the iodo compound **2.46**. Based on steric considerations, the formation of **2.45** was expected to be probably slightly more favoured than **2.43**. However, the respective yields of the two compounds show that **2.43** was the more favoured isomer. These results show that the isomer ratio is not controlled by steric factors, but rather by the higher reactivity of a tosylate attached to a secondary carbon atom because of the greater stability of the substitution transition state.

2.2.2.3 Synthesis of the precursor 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[3-(tributylstannyl)-prop-2-en-1-yloxy]benzamide **2.47**

The precursor **2.47** contains the tributylstannylpropenyl prosthetic group. The starting material required for the introduction of this prosthetic group, (*E*)-3-(tributylstannyl)prop-2-en-1-yl *p*-toluenesulphonate (TBS-PROP-TOS) **2.40**, was synthesised in a two-step synthesis (Scheme 2.3) starting with the treatment of propargyl alcohol **2.38** with tributyltin hydride (*Musachio & Lever, 1992*; *Jung & Light, 1982*), followed by the separation of the geometrical isomers **2.39a** and **2.39b** by means of silica gel column chromatography. The hydroxy group of the *E*-isomer **2.39a** was then tosylated with *p*-toluenesulphonyl chloride according to the strategy of Musachio and Lever (1992). The ^{1}H NMR data of the vinylic and allylic protons of the final product **2.40** corresponded to those quoted in literature (*Musachio & Lever, 1992*), confirming the compound's structure and configuration (Table 2.1).



2.40

TABLE 2.1 Assignment of ^{1}H NMR (300 MHz) data for vinylic and allylic protons in (*E*)-3-(tributylstannyl)prop-2-en-1-yl *p*-toluenesulphonate **2.40**

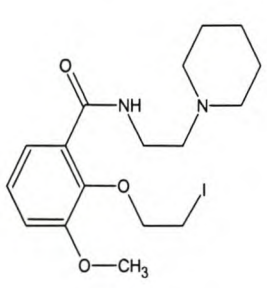
Protons	Assignment of resonances			
	Synthesised		Literature [*Musachio and Lever,1992*]	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
$C\underline{H}_2OTs$	4.56 (2H) dd	5.5, 1.2	4.53 (2H) dd	5.3, 1.2
$C\underline{H}CH_2$	5.88-5.98 (1H) dt	19.1, 5.5	5.90 (1H) dt	19.0, 5.3
$SnC\underline{H}$	6.29-6.36 (1H) dt	19.1, 1.2	6.29 (1H) dt	19.0, 1.2

The tetrabutylammonium phenoxide of 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamides **2.33** was reacted with TBS-PROP-TOS **2.40** under the same conditions employed in the synthesis of the benzamide precursors **2.41**, **2.43** and **2.45**, except for using equimolar amounts of substrate and reagent (Scheme 2.4). The use of an excess of the reagent was unnecessary, as only the tosylate functional group in TBS-PROP-TOS could react with the phenoxide. Chromatography on a silica gel column gave the pure benzamide precursor **2.47** in an overall yield of 85%.

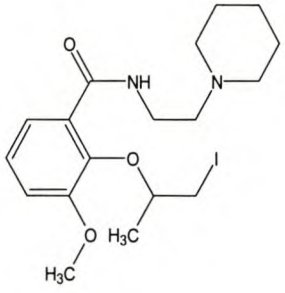
2.2.3 Synthesis of the Iodinated Benzamides **2.42**, **2.44**, **2.46** and **2.48**

In order to obtain "cold" iodinated compounds for NMR and MS characterisation, portions of the precursors were reacted with stable iodide or iodine as shown in Scheme 2.4. In the case of the tosylates **2.41**, **2.43** and **2.45**, the respective precursors were dissolved in acetone and heated in a sealed vial in the presence of a slight molar excess of sodium iodide. In the case of the tributylstannyl precursor **2.47**, the precursor was dissolved in dichloromethane and stirred at room temperature in the presence of an equimolar amount of molecular iodine. The product mixtures were chromatographed over silica gel to give pure 2-(2-iodoethoxy)-3-methoxy-*N*-(2-

piperidin-1-ylethyl)benzamide (**2.42**), 2-(1-iodoprop-2-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide (**2.44**), 2-(2-iodoprop-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)-benzamide (**2.46**) and 2-(3-iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)-benzamide (**2.48**).



2.42



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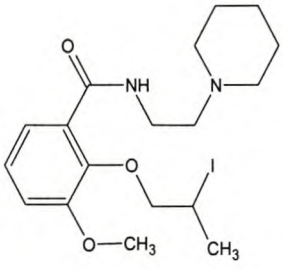

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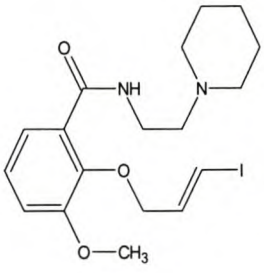
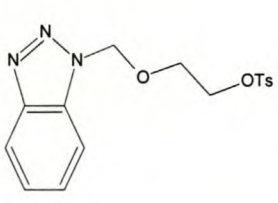


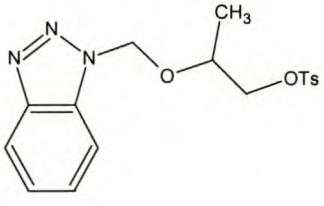
2.48

2.3 SYNTHESIS OF THE HETEROCYCLIC AMINE DERIVATIVES

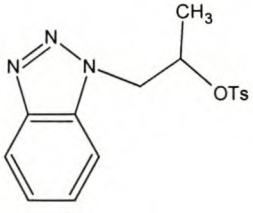
In this section, the synthetic procedures for the various heterocyclic amine derivatives containing the selected prosthetic moieties are discussed. As mentioned before, benzotriazole and 2-methyl-5-nitroimidazole were selected as examples of carriers containing a heterocyclic amine. Both of these compounds are commercially available. The benzotriazole derivatives 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65**, 1-[(1-*p*-toluenesulphonyloxy-prop-2-yloxy)methyl]benzotriazole **2.69**, 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73** and 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.77** were selected as precursors for radioiodination:



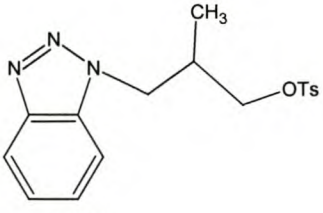
2.65



2.69

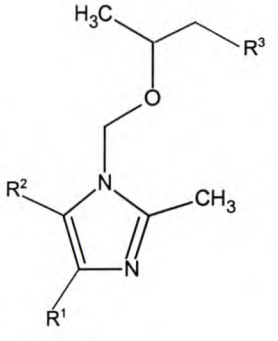


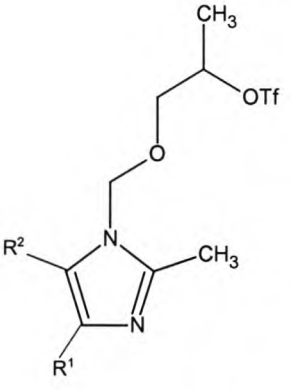
2.73

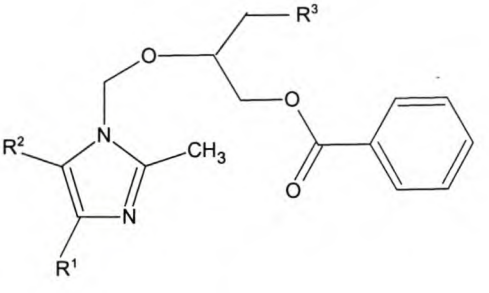


2.77

In the nitroimidazole series, the 2-methyl-5-nitroimidazole derivatives 2-methyl-5-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.84**, 2-methyl-5-nitro-1-[(1-trifluoro-methanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.86**, 2-methyl-5-nitro-1-[(2-trifluoro-methanesulphonyloxyprop-1-yloxy)methyl]imidazole **2.91**, 1-[(1-benzoyloxy-3-trifluoro-methanesulphonyloxyprop-2-yloxy)methyl]-2-methyl-5-nitroimidazole **2.96** and 1-[(1-benzoyl-oxy-3-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]-2-methyl-5-nitroimidazole **2.98** were initially considered as precursors. However, based on steric considerations, it was expected that the *N*-3 atom on the 2-methyl-5-nitroimidazole heterocyclic ring would probably be preferentially alkylated. This would result in 2-methyl-4-nitroimidazole derivatives. The *N*-alkylated nitroimidazole target compounds prepared in this study were therefore in fact the 2-methyl-4-nitroimidazole derivatives **2.83, 2.85, 2.90**, **2.95** and **2.97** respectively. This will be discussed in greater detail in paragraph 2.3.2.







$R^1 = NO_2$; $R_2 = H$; $R^3 = OTs$ **2.83**
$R^1 = H$; $R^2 = NO_2$; $R^3 = OTs$ **2.84**
$R^1 = NO_2$; $R^2 = H$; $R^3 = OTf$ **2.85**
$R^1 = H$; $R^2 = NO_2$; $R^3 = OTf$ **2.86**

$R^1 = NO_2$; $R^2 = H$ **2.90**
$R^1 = H$; $R^2 = NO_2$ **2.91**

$R^1 = NO_2$; $R^2 = H$; $R^3 = OTf$ **2.95**
$R^1 = H$; $R^2 = NO_2$; $R^3 = OTf$ **2.96**
$R^1 = NO_2$; $R^2 = H$; $R^3 = OTs$ **2.97**
$R^1 = H$; $R^2 = NO_2$; $R^3 = OTs$ **2.98**

To obtain the iodinated analogues of all of these compounds, the substitution of their tosylate or triflate groups with iodine was envisaged. The 1-benzoyloxy group in the iodinated analogues of 1-[(1-benzoyloxy-3-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.95** and 1-[(1-benzoyloxy-3-*p*-toluenesulphonyloxyprop-2-

yloxy)methyl]-2-methyl-4-nitroimidazole **2.97** were intended to be converted to a hydroxy group (see Section 2.3.4).

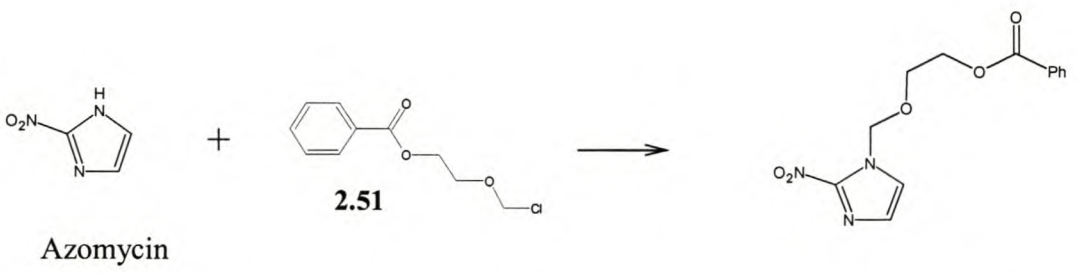
The prosthetic moieties in the benzotriazole-derived precursors 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65** and 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69** are of a similar nature to those present in the benzamides 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)ethoxy]benzamide **2.41** and 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[1-(*p*-toluenesulphonyloxy)prop-2-yloxy]benzamide **2.43** respectively, while those in 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73** and 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.77**, both having branched chains, lacked a β-oxygen atom. The radioiodinated analogues of **2.73** and **2.77** were selected to serve as reference compounds, i.e. **2.74** and **2.78** (similar to the model compound 1-iodo-2-phenylethane **2.30**). Their radiochemical stabilities were to be compared with those of the radioiodinated analogues of 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69**, i.e. **2.70**, and 2-methyl-4-nitro-1-[(2-trifluoromethanesulphonyloxyprop-1-yloxy)methyl]imidazole **2.90**, i.e. **2.92**, both having β-oxygen atoms in their branched chains, in order to determine the influence of branching in the prosthetic group on the radiochemical stability. This exercise was expected to give an approximate indication of the contribution of steric factors, caused by branching, towards the expected higher stability of the radioiodinated analogue of 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69**, in contrast to the radioiodinated analogue of 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65**, i.e. **2.66b**.

The prosthetic group in the nitroimidazole precursor 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.83** was identical to that in the benzotriazole precursor **2.69**, while the prosthetic group in 1-[(1-benzoyloxy-3-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.95** or its tosylated analogue **2.97** resembled the prosthetic group of 1-[2-[^{18}F]fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole **1.32** reported in Chapter 1. As methods for

the synthesis of **1.32** or its intermediate 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole **1.31** are not described in the literature, novel methods had to be developed during the course of this work to synthesise the intermediates required for the preparation of **2.95** and **2.97**.

The prosthetic group in 2-methyl-4-nitro-1-[(2-trifluoromethanesulphonyloxyprop-1-yloxy)methyl]imidazole **2.90** resembled the one in 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)prop-1-yloxy]benzamide **2.45**. The triflate precursors 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85, 2.90** and **2.95** differed from all the others with respect to the nature of their leaving groups. According to Fleet (1989), triflates are preferred to tosylates as leaving groups for nucleophilic substitutions at secondary carbons in carbohydrates. The reason is that the β-oxygen atom slows down S_N reactions dramatically, and that triflate is an extremely good leaving group (*Fleet, 1989*; *Wheeler et al., 1987*). Therefore the triflate was used as leaving group in precursor **2.90**. In addition to the tosylate precursor 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.83**, its triflate analogue **2.85** was also prepared in order to compare their respective reactivities towards nucleophilic displacement with iodide. Because the triflate precursor **2.95** was only used for the *in situ* synthesis of the "cold" iodinated compound **2.99**, while its more stable tosylate analogue **2.97** was used in radioiodinations, both of these precursors were prepared. Tosylate **2.97** was prepared according to the method of Wada *et al.* (2000), which is similar to the tosylation method used by Argentini *et al.* (1981).

A different strategy had to be followed in order to attach the various prosthetic moieties to the nitrogen atom in the synthesis of the β-oxygen-containing precursors **2.65**, **2.69**, **2.83**, **2.85**, **2.90**, **2.95** and **2.97**. In contrast to the benzamide derivatives in which the carrier compound already contained the β-oxygen atom, this atom had to be incorporated into the prosthetic moieties used for the synthesis of the amine derivatives. It was decided to follow the method of Srivastava *et al.* (1991) in which a nitroimidazole (azomycin) was condensed with the alkylating agent 2-chloromethoxyethyl benzoate **2.51**, according to Scheme 2.5.



Azomycin

Scheme 2.5 Condensation of azomycin with 2-chloromethoxyethyl benzoate **2.51**

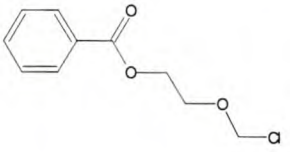
Hydrolysis of the ester function of this condensation product resulted in the formation of an OH group two carbons removed from the methyloxy oxygen atom that served as the β-oxygen atom. Various methods are available for the substitution of an OH group with "cold" iodine, amongst others iodination with the reagent methyltriphenoxyphosphonium iodide. Isotope exchange radiolabelling of the "cold" iodinated compound gives the radioiodinated compound (*Srivastava et al., 1991*). In the present investigation it was decided to rather convert the OH group to a tosylate or other good leaving group in order to obtain the same type of precursors as those prepared from the benzamides.

Initially a major problem in the synthesis of the mentioned derivatives was that the appropriate alkylating agents were not commercially available. Srivastava *et al.* (1991) did not report a method for the synthesis of 2-chloromethoxyethyl benzoate **2.51**. Methods therefore had to be developed to synthesise all the relevant alkylating agents. A feasible general strategy was to chloromethylate the free hydroxy group of the monobenzoate ester of a glycol. The following section describes attempts to synthesise these reagents.

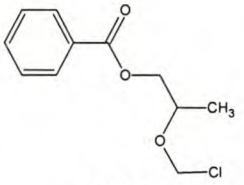
2.3.1 Synthesis of Alkylating Agents

The following alkylating agents were synthesised in order to prepare the various *N*-alkylated heterocyclic amine derivatives: 2-chloromethoxyethyl benzoate **2.51**, 2-chloromethoxyprop-1-

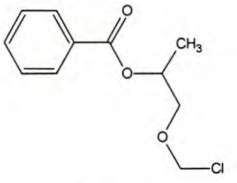
yl benzoate **2.56**, 1-chloromethoxyprop-2-yl benzoate **2.57** and 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61**.



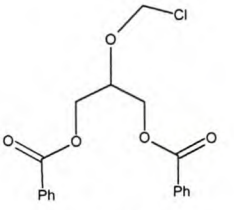
2.51



2.56



2.57

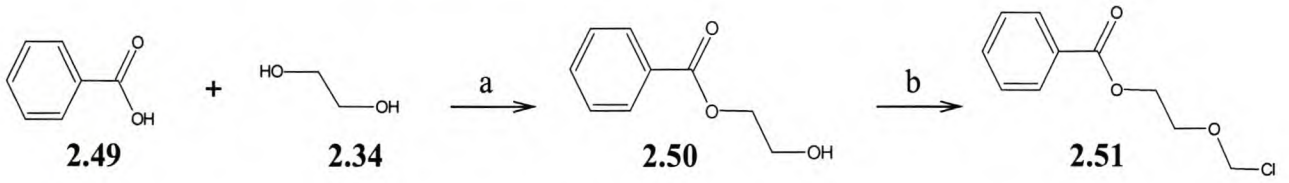


2.61

2.3.1.1 Synthesis of 2-chloromethoxyethyl benzoate **2.51**

The synthetic pathway is given in Scheme 2.6. The starting material for this synthesis was 2-hydroxyethyl benzoate **2.50**, prepared by heating a mixture of ethylene glycol **2.34** and benzoic acid **2.49** in a molar ratio of 3:1, respectively, in the presence of a catalytic amount of concentrated sulphuric acid and 3Å molecular sieves. After work-up, TLC (Method 8) showed only a trace of an impurity suspected to be ethylene dibenzoate in the product **2.50**. The product **2.50** was pure enough to use without further purification. The chloromethyl ether was initially prepared according to the method of Hakimelahi *et al.* (1987). According to this method, aqueous hydrochloric acid, adsorbed on silica gel, converts alcohols in aprotic solvents to the corresponding chloromethyl ethers in the presence of an aldehyde. The chloromethylation is carried out in chloroform at room temperature, using the dried HCl/silica gel mixture and 1,3,5-trioxane as a source of aldehyde. However, despite numerous attempts, 2-hydroxyethyl benzoate **2.50** could not be converted to its chloromethyl ether by using this method. TLC- and HPLC analysis of the reaction mixtures showed the formation of products, but according to qualitative tests for halogen (*Furniss et al., 1989*), not one of the products isolated on a silica gel column by chromatography contained a chlorine atom. Attempts to alkylate amines with either of these products also failed. Alternative methods for chloromethylation were subsequently considered. The method described by Guédin-Vuong and

Nakatani (1986) seemed promising. This method, designed for the chloromethylation of long-chain alcohols, involves the reaction of the alcohol with paraformaldehyde and gaseous hydrogen chloride at a low temperature of not higher than -10°C (Scheme 2.6). According to the authors, it is imperative that the temperature be maintained below -10°C to avoid a dismutation leading to a mixture of the symmetrical acetal and methylene chloride. It has been stated that the product should not be distilled or chromatographed over silica gel, which possibly partially explains the failure of the attempts at using the method proposed by Hakimelahi *et al.* Guédin-Vuong and Nakatani's method gave the target chloromethyl ether **2.51** in high yields of 93-100%. The product showed a positive halogen test. Due to its instability, it was not purified or characterized. It was either used on the day of its preparation, or stored in methylene chloride at about -10°C for a few days only.



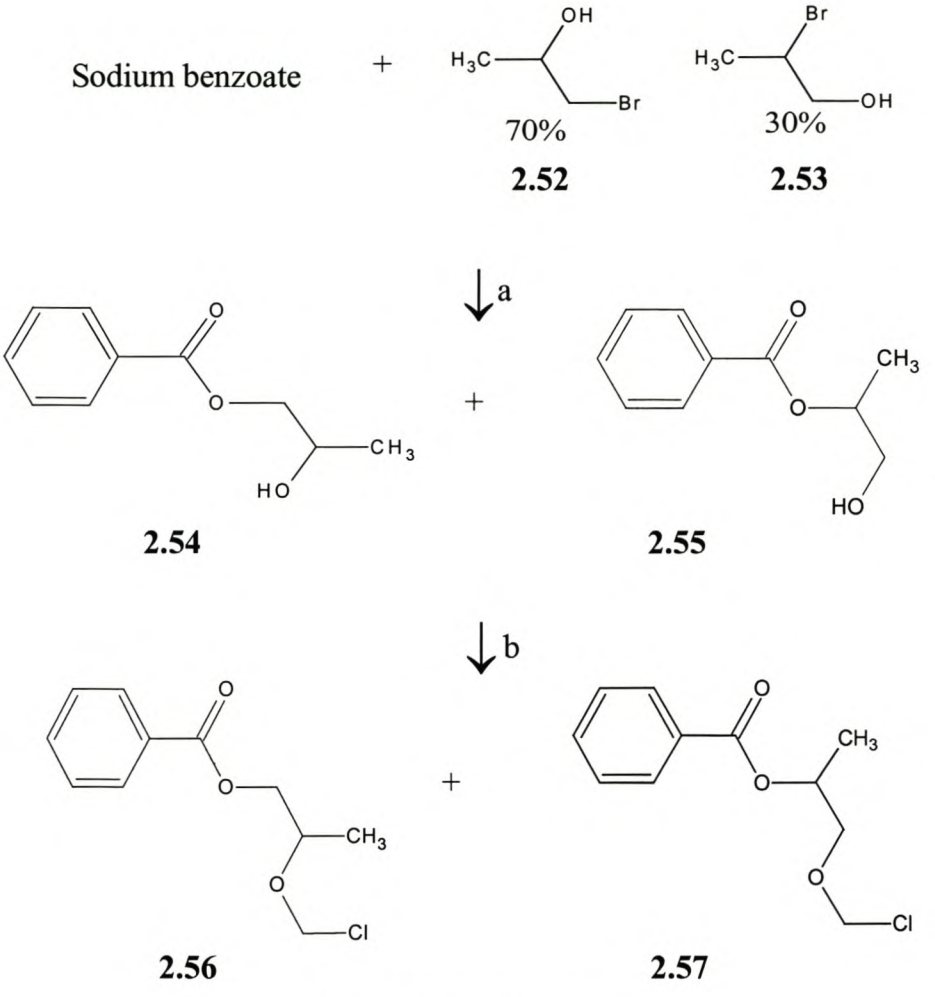
2.49 **2.34** **2.50** **2.51**

a. H_2SO_4
b. $(CH_2O)_n$, HCl(g), -10°C

Scheme 2.6 Synthesis of 2-hydroxyethyl benzoate **2.50** and 2-chloromethoxyethyl benzoate **2.51**

2.3.1.2 Synthesis of 2-chloromethoxyprop-1-yl benzoate **2.56**

The synthetic pathway for the synthesis of 2-chloromethoxyprop-1-yl benzoate **2.56** from the corresponding monoester 2-hydroxyprop-1-yl benzoate **2.54** is outlined in Scheme 2.7. At first glance, propane-1,2-diol appeared to be the obvious starting material for the synthesis of the monoester **2.54**, but the regioselective esterification of the primary OH group was expected to be problematic. Instead, it was decided to use a halohydrin such as 1-bromo-2-propanol **2.52**, in which the halogen could be selectively substituted with a benzoate group in a dipolar aprotic solvent (*March, 1992*). Unfortunately, the best quality commercially available 1-bromo-2-



a. 60°-130°C, DMF or HMPA
b. $(CH_2O)_n$, HCl (g), -10°C

Scheme 2.7 Synthesis of 2-chloromethoxyprop-1-yl benzoate **2.56** containing the isomeric impurity 1-chloromethoxyprop-2-yl benzoate **2.57**

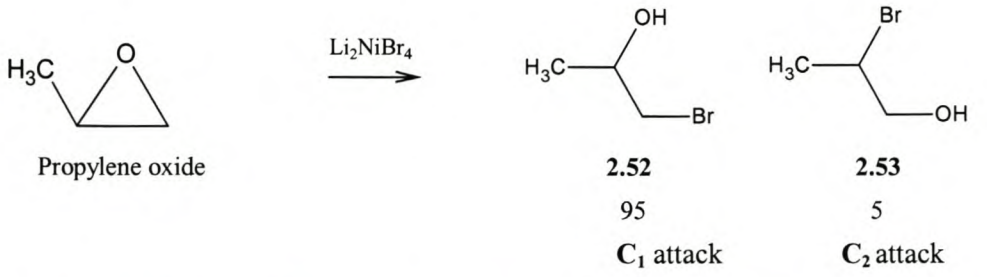
propanol **2.52** contains about 30% of the isomeric 2-bromo-1-propanol **2.53** as an impurity. It was anticipated that all the intermediate products would therefore be contaminated with isomeric impurities such as 1-hydroxyprop-2-yl benzoate **2.55**. Some exploratory experiments to synthesise 2-hydroxyprop-1-yl benzoate **2.54** were nevertheless initially carried out with the impure material. It was, however, also attempted to synthesise 1-bromo-2-propanol of a better quality than the commercial material. The exploratory experiments, using the impure material,

will now be discussed, followed by a discussion of the synthesis of the better quality 1-bromo-2-propanol **2.52** and its conversion to pure 2-hydroxyprop-1-yl benzoate **2.54**.

Dry sodium benzoate was heated with the mixture of bromohydrins **2.52** and **2.53** in dimethylformamide (DMF) at 120°-130°C. HMPA was recommended as solvent because of its alleged superior efficiency as solvent for esterification by halogen displacement (*March, 1992; Shaw et al., 1973; Pfeffer & Silbert, 1976*), but the dipolar aprotic DMF was used instead because it is cheaper and more readily available. The assumption was that both isomers present in the bromohydrin mixture would react quantitatively with the benzoate, resulting in a product containing about 30% of the undesired isomer 1-hydroxyprop-2-yl benzoate **2.55**. Work-up and silica gel chromatography gave a product (68%) that showed only a single spot on TLC (Method 7). On HPLC analysis of this product [Method 3(a)], two well resolved peaks were obtained in a ratio of 65:35. The peak with the shorter retention time (the more polar component) was the most prominent and was deemed to be the desired 2-hydroxyprop-1-yl benzoate **2.54**. The esterification of propane-1,2-diol with benzoic acid under acidic conditions, using a three-fold excess of the diol to suppress the formation of the di-ester, incidentally also resulted in the formation of **2.54** and **2.55** in approximately the same ratio. As the ester could not be crystallised, it was impossible to separate the two isomers by crystallisation. Furthermore, it was unlikely that they would be separable by column chromatography because TLC on silica gel gave only one spot. At this stage it was decided to continue the synthesis of the subsequent intermediate products, using the 65%-pure 2-hydroxyprop-1-yl benzoate **2.54**, in the hope that steric influences would promote the elimination of the undesired isomers.

Investigation of the synthesis of pure 1-bromo-2-propanol **2.52** was started by exploring the method proposed by Dawe *et al.* (1984) and Bonini and Righi (1994). These authors claim that the reaction of propylene oxide with freshly prepared Li_2NiBr_4 in dry THF at room temperature should produce the two isomers **2.52** and **2.53** in a ratio of *ca.* 95:5 respectively, according to Scheme 2.8. Several attempts to synthesise **2.52** by using this method gave impure products. Using lower reaction temperatures and different work-up procedures again gave isomer ratios

of between 79:21 and 86:14. This synthetic route to the 1-bromo-2-propanol **2.52** was therefore abandoned.



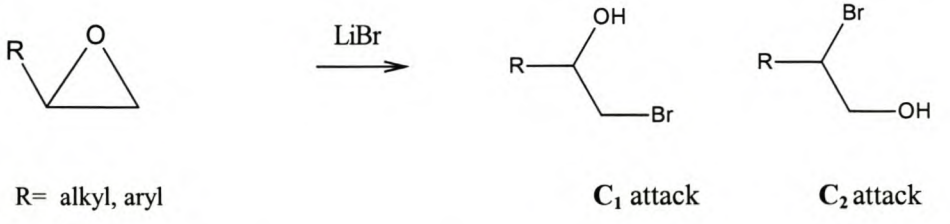
Propylene oxide

2.52 95 C_1 attack

2.53 5 C_2 attack

Scheme 2.8 Synthesis of 1-bromo-2-propanol **2.52** according to Dawe *et al.* (1984) and Bonini and Righi (1994).

Bonini *et al.* (1992) claimed a ratio of C_1:C_2 of between 75:25 and 99:1 for different unspecified halogen derivatives using lithium bromide as the source of bromide, Amberlyst 15 as the acidic catalyst and dry acetonitrile as the solvent, according to Scheme 2.9. However, several variations of the method of Bonini *et al.* (1992) did not yield better than 75:25 ratios.



R= alkyl, aryl

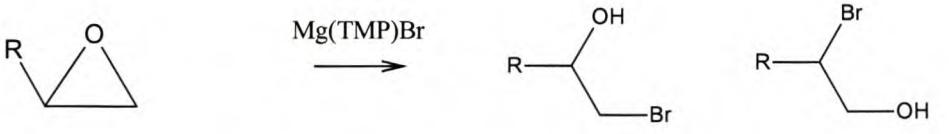
C_1 attack

C_2 attack

Scheme 2.9 Synthesis of 1-bromo-2-hydroxy derivatives according to Bonini *et al.* (1992)

The synthesis of 1-bromo-2-decanol according to Eisch *et al.* (1992), as depicted in Scheme 2.10, gave this compound in a high degree of purity. Unfortunately the long-chain 1,2-epoxydecane was the only starting material that was used by Eisch *et al.* to demonstrate the selectivity of this synthesis. A Grignard reagent is treated with a non-nucleophilic amine, and

the resulting compound is reacted with an epoxide. Although it is difficult to remove the amine used in this synthesis, 2,2,6,6-tetramethylpiperidine, from the product, it was possible to synthesise 1-bromo-2-propanol **2.52** in a yield of 41% and a high degree of purity of more than 99% with the use of this method. Extended storage and distillation of the crude product at high temperatures should be avoided because the target product can react with 2,2,6,6-tetramethylpiperidine and can eventually produce the starting epoxide. Most of the unreacted amine was removed from the product by means of the ammonium chloride solution that is used to hydrolyse the intermediates. The removal of THF from 1-bromo-2-propanol is also difficult because the boiling point of the target compound is relatively low. Substituting diisopropylamine for the expensive 2,2,6,6-tetramethylpiperidine also gave an acceptable product in a yield of 43%.



R = 1-octyl TMP = 2,2,6,6-tetramethylpiperidine *100* *0*

Scheme 2.10 Synthesis of 1-bromo-2-decanol according to Eisch *et al.* (1992)

GC-MS analysis of the product (Method 1) confirmed the identity of 1-bromo-2-propanol. The molecular ions (*m/z* 138 and 140) arising from the two stable bromine isotopes were present in a low concentration (0.5% each), but the *m/z* peak of 45, characteristic of secondary alcohols, was present as base peak. According to GC analysis (Method 1), the purest fraction showed an overall purity[5] of approximately 85% and contained only 0.3% 2-bromo-1-propanol. Using this fraction as starting material, an additional synthesis of 2-hydroxyprop-1-yl benzoate **2.54** was carried out in an attempt to prepare an isomer-free product. HMPA, instead of DMF, was used

[5] The solvent is included in calculating the overall purity. In this case, for example, the product contained *ca.* 15% solvent and co-eluting volatile material

as solvent for this synthesis. Preliminary pilot experiments had indicated that the reaction proceeded very slowly at room temperature, while prolonged heating at higher temperatures (70°-100°C) resulted in the formation of increasing amounts of the undesired 1-hydroxyprop-2-yl benzoate **2.55**, presumably due to rearrangement. Work-up and silica gel chromatography of a reaction product obtained under optimised reaction conditions (reaction temperature = 60°C, reaction time = 2 hours) gave compound **2.54** in an isomeric purity of 97% and a yield of approximately 58%.

2-Chloromethoxyprop-1-yl benzoate **2.56** was prepared from both the 65%- and 97%-pure 2-hydroxyprop-1-yl benzoate **2.54**. Guédin-Vuong and Nakatani's method (1986) for the synthesis of 2-chloromethoxyethyl benzoate **2.51** was utilised. As before, this method proved to be very successful and gave the product in quantitative yield. This chloromethyl ether was used without purification in the next step of the preparation of the *N*-alkylated heterocyclic amine.

2.3.1.3 Synthesis of 1-chloromethoxyprop-2-yl benzoate **2.57**

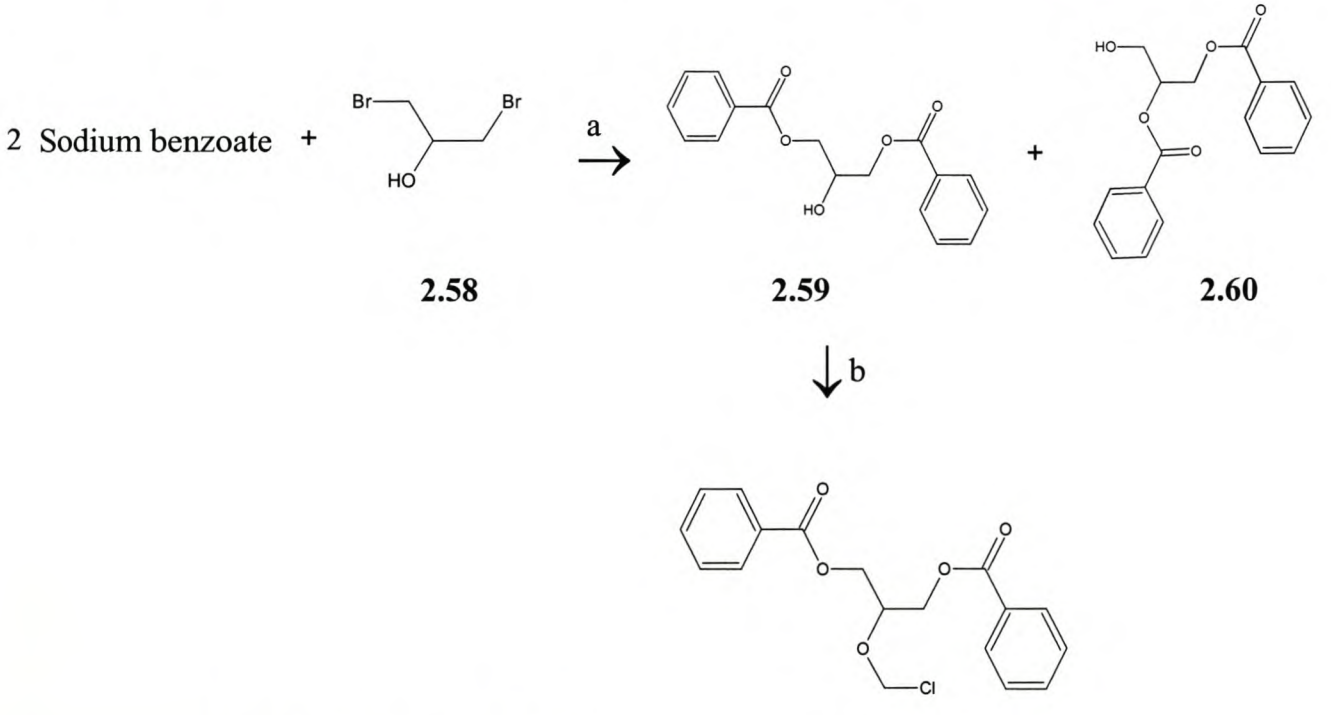
2-Bromo-1-propanol **2.53** was needed as the halohydrin source for the synthesis of the monoester 1-hydroxyprop-2-yl benzoate **2.55**, the required starting material for the synthesis of 1-chloromethoxyprop-2-yl benzoate **2.57**, as shown in Scheme 2.7. Since 2-bromo-1-propanol **2.53**, the contaminating isomer present in the commercial 1-bromo-2-propanol **2.52**, is not commercially available, it had to be synthesised. The strategy chosen for the synthesis of this bromohydrin was based on the reduction of 2-bromo-propionic acid to the corresponding alcohol. Lithium aluminiumhydride ($LiAlH_4$) is mostly used as reducing agent for the reduction of carboxylic acids or esters to alcohols, but aluminium hydride was preferred in order to prevent dehydrobromination as a possible side reaction. This agent was generated *in situ* by treating $LiAlH_4$ with concentrated sulphuric acid at 0° to 5°C, according to the method of Shah *et al.* (1996). The 2-bromopropionic acid was treated with the resulting H_2SO_4/AlH_3 mixture. Work-up gave a product in low yield (approximately 20%). GC analysis (Method 1) of this

product showed a major peak with a retention time similar to that of the smaller peak in the GC chromatogram of the commercial 1-bromo-2-propanol, i.e. the contaminant 2-bromo-1-propanol, as well as a smaller peak with a retention time similar to that of the major peak in the commercial 1-bromo-2-propanol. This suggested that the required product (2-bromo-1-propanol) had been formed. The ratio of 2-bromo-1-propanol **2.53** to 1-bromo-2-propanol **2.52** in this product was approximately 95:5, while its overall purity was approximately 70% (GC). It was not distilled to avoid possible isomerisation.

This preparation of 1-hydroxyprop-2-yl benzoate **2.55** from the bromohydrin **2.53** was carried out in DMF. Experiments were carried out to find the optimum reaction conditions such as reaction temperature and reaction time. HPLC analysis of the reaction mixtures [Method 3(a) or 3(b)] showed the presence of a higher than expected amount of 2-hydroxyprop-1-yl benzoate **2.54**, formed as a result of the presence of 1-bromo-2-propanol **2.52** in the starting material **2.53**. In one experiment, the reaction temperature was maintained between 100° and 117°C. The ratio of **2.55**:**2.54** changed from = 82:18 to 66:34 over a period of approximately six hours. In another experiment, in which the reaction temperature was maintained at 100°C over a period of approximately 2.5 hours, the final ratio of **2.55**:**2.54** was 80:20. These results suggested that some rearrangement had taken place during prolonged heating, as the starting 2-bromo-1-propanol **2.53** contained only 5% 1-bromo-2-propanol **2.52**. The reason for the extended heating was to optimise the yield of the product. Unfortunately, optimum isomeric purity had to be sacrificed in order to obtain an acceptable yield. No further attempts were made to optimise reaction conditions in order to ensure optimum isomeric purity. Crude reaction products were worked up and chromatographed over silica gel. Purified products obtained from various experiments were combined to give a final product containing the two monoesters **2.55** and **2.54** in a ratio of 70:30. Despite this lower isomeric purity of 1-hydroxyprop-2-yl benzoate **2.55**, it was nevertheless decided to use it as substrate for the preparation of chloromethyl ether **2.57** according to the method of Guédin-Vuong and Nakatani (1986).

2.3.1.4 Synthesis of 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61**

This reagent, required for the alkylation of 2-methyl-5-nitroimidazole, was synthesised according to the reaction pathway depicted in Scheme 2.11. The starting material 1,3-dibromo-2-propanol **2.58** is commercially available in a purity of 95%. It was heated in HMPA or DMF with a two- to three-fold molar excess of sodium benzoate to produce 1,3-dibenzoyloxy-2-propanol **2.59**. Firstly, in an adaptation of the method described by Shaw *et al.* (1973), sodium benzoate was formed *in situ* by treating benzoic acid with sodium hydride in hexamethylphosphoramide (HMPA). 1,3-Dibromo-2-propanol **2.58** was then added to the mixture. In contrast to the method described by Shaw, in which the reaction was carried out at room temperature using an excess of the halide, the synthesis under investigation had to be carried out at an elevated temperature (110°C) in order to dissolve all of the benzoate.



2.58 **2.59** **2.60** **2.61**

a. 110°-165°C, DMF or HMPA
b. $(CH_2O)_n$, HCl (g), -8°C

Scheme 2.11 Synthesis 1,3-dibenzoyloxy-2-propanol **2.59** and 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61**

In a second procedure, in which a mixture of previously prepared sodium benzoate and DMF was used, a higher reaction temperature (165°C) was needed to dissolve the benzoate. According to HPLC-analysis (Method 4) of the products obtained in these experiments, the products contained two major constituents in a ratio of ca 3:7. As the starting material was 95% pure, the formation of an impurity in a concentration of 30% was somewhat unexpected. The impurity was presumed to be the hydroxydibenzoate **2.60** formed by a rearrangement analogous to the formation of 2-hydroxyprop-1-yl benzoate **2.54** as a by-product of the synthesis of 1-hydroxyprop-2-yl benzoate **2.55** described in Section 2.3.1.3.

The products obtained in all of these experiments were combined and purified by column chromatography over silica gel. In contrast to the mixture of the monoesters 2-hydroxyprop-1-yl benzoate **2.54** and 1-hydroxyprop-2-yl benzoate **2.55**, the two constituents of this mixture, 1,3-dibenzoyloxy-2-propanol **2.59** and 2,3-dibenzoyloxy-1-propanol **2.60**, could be fairly well separated by means of silica gel chromatography, although a large fraction of the eluted material contained both compounds. The less polar, major constituent was isolated in a 24% yield and a purity of 95%. The more polar, minor constituent was isolated in a yield of 3%, but in a similar purity. ^{1}H NMR data (TABLE 2.2) confirmed the less polar compound to be 1,3-dibenzoyloxy-2-propanol **2.59**. The ^{1}H NMR spectrum of the more polar compound is in good agreement with the proposed structure, 2,3-dibenzoyloxy-1-propanol **2.60**. While the chemically equivalent methylene protons in **2.59** have the same chemical shift, the chemical shifts of the non-equivalent methylene protons in **2.60** differ by approximately 0.7 ppm, with those geminal to the hydroxyl group resonating at higher field. Likewise, the chemical shift of the methine proton at δ 5.5 in the spectrum of the hydroxydiester **2.60**, being geminal to a carboxylic ester oxygen atom, resonates at a lower field than the analogous proton of hydroxydiester **2.59** (δ 4.4).

The reaction conditions used for the preparation of the alkylating agents **2.51**, **2.56** and **2.57** were also employed to synthesise the alkylating agent 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61** from 1,3-dibenzoyloxy-2-propanol **2.59**. However, the preparation

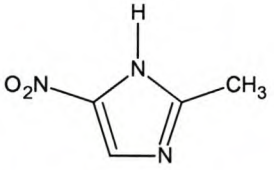
TABLE 2.2 ^{1}H NMR (300 MHz) data for the hydroxydibenzoates **2.59** and **2.60**

Protons	PhCOO-CH_2-CH(OH)-CH_2-OCOPh **2.59** δ (ppm)	HO-CH_2-CH(OCOPh)-CH_2-OCOPh **2.60** δ (ppm)
O<u>H</u>	2.91 (1H) d	2.48 (1H) broad m
C<u>H</u>OH	4.38 - 4.44 (1H) m	-
$C\underline{H_2}OH$	-	3.98 (2H) d
$C\underline{H_2}OCO$	4.49 - 4.59 (4H) dd	4.70 (2H) d
C<u>H</u>OCO	-	5.48 - 5.55 (1H) quintet

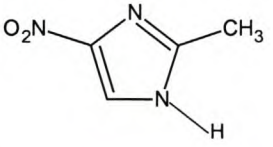
of **2.61** was much less successful than that of the others. The purity of this type of chloromethyl ether cannot be easily determined chromatographically because of its instability (*Guédin-Vuong & Nakatani, 1986*). However, an idea of its purity can be obtained by observing the yield of the condensation product (e.g. 1-[(1,3-dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.93** obtained by the reaction of the chloromethyl ether (e.g. **2.61**) with an amine such as 2-methyl-5-nitroimidazole). A poor condensation yield can most probably be attributed to an impure chloromethyl ether. The yields of the various condensation products, using the various chloromethyl ethers, are discussed in Section 2.3.2.1.1. Alkylated products in modest to fairly good yields were given by 2-chloromethoxyethyl benzoate **2.51**, 2-chloromethoxyprop-1-yl benzoate **2.56** and 1-chloromethoxyprop-2-yl benzoate **2.57**. In contrast to these yields, 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61** gave the alkylated amine **2.93** in widely varying yields, ranging from very poor to modest. This phenomenon can probably be explained in terms of possible steric hindrance caused by the two geminal benzoyloxymethyl groups at the secondary hydroxy group in the precursor, 1,3-dibenzoyloxy-2-propanol **2.59**, which might impede the ease of chloromethylation at this reaction site.

2.3.2 Preparation of *N*-alkylated Amines

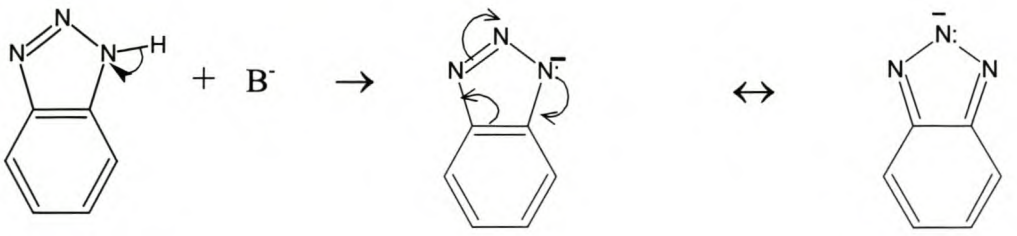
In order to synthesise the amine precursors, a nitrogen atom had to be alkylated in the heterocyclic rings of benzotriazole and 2-methyl-5-nitroimidazole, respectively. Initially, it was not clear at which nitrogen atom in the heterocyclic ring of benzotriazole alkylation would preferentially occur. In the case of 2-methyl-5-nitroimidazole, it was easier to predict the preferred site of *N*-alkylation. Tautomer II, 2-methyl-4-nitroimidazole, would not exist in significant quantities because of the stabilisation of tautomer I, 2-methyl-5-nitroimidazole, through hydrogen bonding between the NH proton and the negatively polarised oxygen atom of the adjacent nitro group. However, after abstraction of the proton from the nitrogen atom with base, there will be no difference in the stability between the two resonance structures (shown in Scheme 2.12), and the site of *N*-alkylation should be ruled by a steric influence exerted by the nitro group on the adjacent N-1 atom of 2-methyl-5-nitroimidazole. *N*-alkylation of this compound would therefore probably preferentially occur at the more accessible N-3 atom. In such a case, the major *N*-alkylated compounds formed would be 2-methyl-4-nitroimidazole derivatives.



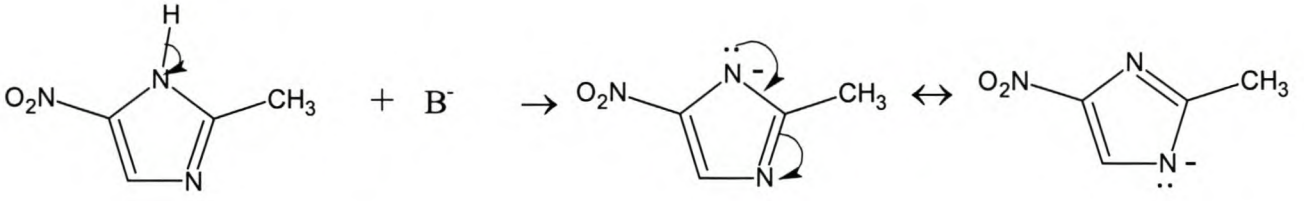
2-Methyl-5-nitroimidazole (Tautomer I)



2-Methyl-4-nitroimidazole (Tautomer II)



Benzotriazole **2.62** **2.62** (N1) **2.62** (N2)



2-Methyl-5-nitroimidazole **2.79** **2.79** (N1) **2.79** (N3)

Scheme 2.12 Resonance structures of the anion that is formed by abstraction of a proton from the amine nitrogen atom of benzotriazole and 2-methyl-5-nitroimidazole

The site of *N*-alkylation in the various molecules was confirmed by ^{13}C NMR spectral data obtained from the major *N*-alkylated compounds formed, as well as by those obtained from by-products. Due to the element of symmetry in benzotriazole **2.62** (anion N2), one would expect that there would be only three aromatic ^{13}C signals in the ^{13}C NMR spectra of all its alkylated products. In practice, all the isolated benzotriazole derivatives synthesised in this work exhibited six ^{13}C signals in the aromatic region of the spectra, suggesting that they had been derived from **2.62** (N1) in which all six the aromatic carbons are non-equivalent. Only one benzotriazole alkylated by-product was isolated, and as its ^{13}C NMR spectrum had indeed only three aromatic carbon signals, it was concluded that it had the structure 2-(2-hydroxyprop-1-yl)benzotriazole **2.72** (see the preparation of compound **2.71** and the isolation of the by-product **2.72** in paragraph 2.3.2.2).

A comparison of the chemical shift values of the carbon signals in the ^{13}C spectra of the *N*-alkylated nitroimidazole compounds synthesised in this work and those in the spectra of authentic 2-methyl-5-nitroimidazole and 2-methyl-4-nitroimidazole derivatives, confirmed the earlier speculations on the preferred site of alkylation in this molecule. These data are shown in Table 2.3.

TABLE 2.3 Comparison of ^{13}C NMR data of a few *N*-alkylated nitroimidazole derivatives synthesised in this study with those of *reference compounds

Compound	^{13}C NMR chemical shift values of heterocyclic ring carbon atoms δ (ppm)
1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80**	119.56, 145.46, 147.08
1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-5-nitroimidazole **2.81**	133.35, 138.36, 152.05
1-[(2-benzoyloxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.88**	119.64, 145.56, 146.87
1-[(1,3-dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.93**	119.72, 145.54, 146.82
By-product of compound **2.93**	133.45, 138.27, 152.06
*1-(2-chloroethyl)-2-methyl-5-nitroimidazole	133.51, 138.19, 151.45
*1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole	132.79, 138.29, 151.79
*3-(2-methyl-4-nitroimidazol-1-yl)propionic acid	121.84, 145.16, 145.27
*1-(2-cyanoethyl)-2-methyl-4-nitro-imidazole	121.85, 145.22, 145.47

*Data obtained from the Aldrich Library of ^{13}C and ^{1}H FTNMR spectra, Edition I, Volume 3 (1993).

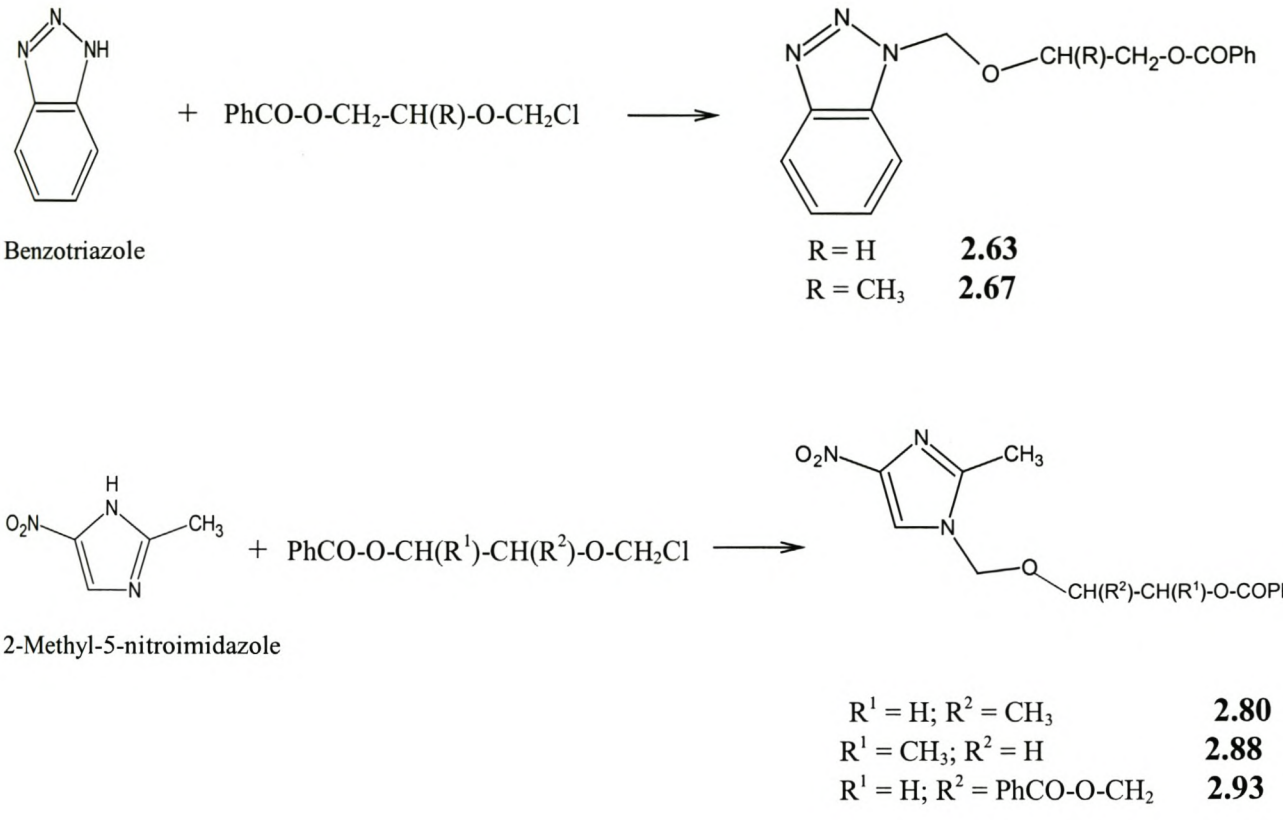
The data in Table 2.3 confirm that the major products obtained in the *N*-alkylation of 2-methyl-5-nitroimidazole in this work had formed as a result of *N*-alkylation at the N-3 atom, leading to the formation of 2-methyl-4-nitroimidazole derivatives (e.g. **2.80**, **2.88** and **2.93**). The ^{13}C NMR chemical shift data of the by-products isolated in the synthesis of compounds **2.93** and **2.80** (compound **2.81**), displayed in Table 2.3, are in good agreement with those of 2-methyl-5-nitroimidazole derivatives. As the main emphasis in this work was placed on the structures of

the aliphatic prosthetic groups rather than those of the carrier molecules, no attempts were made to isolate or prepare more of the 2-methyl-5-nitroimidazole derivatives.

Various reaction conditions are used for *N*-alkylation. For the condensation of 2-nitroimidazole with 2-benzoyloxyethoxymethyl chloride, Srivastava *et al.* (1991) used dimethylformamide as solvent and triethylamine as base. For the condensation of the same substrate with 2-bromoethyl-2-tetrahydropyranyl ether, Jerabek *et al.* (1986) used *N,N*-dimethylacetamide (DMA) as solvent, sodium methoxide as base, and a reaction temperature of 90°C. Milder reaction conditions were employed by Waterhouse *et al.* (1996) for the *N*-alkylation of 4-(4-cyanophenoxymethyl)piperidine with propargyl bromide. The reaction was carried out at room temperature, using dichloromethane as solvent and potassium carbonate as base. Acetonitrile is also often used as solvent for *N*-alkylations (*Chi et al., 1986*; *Shi et al., 1999; Yasunaga et al., 1998*). For the purpose of this work, the most suitable reaction conditions were selected, depending on the type of alkylating agent used.

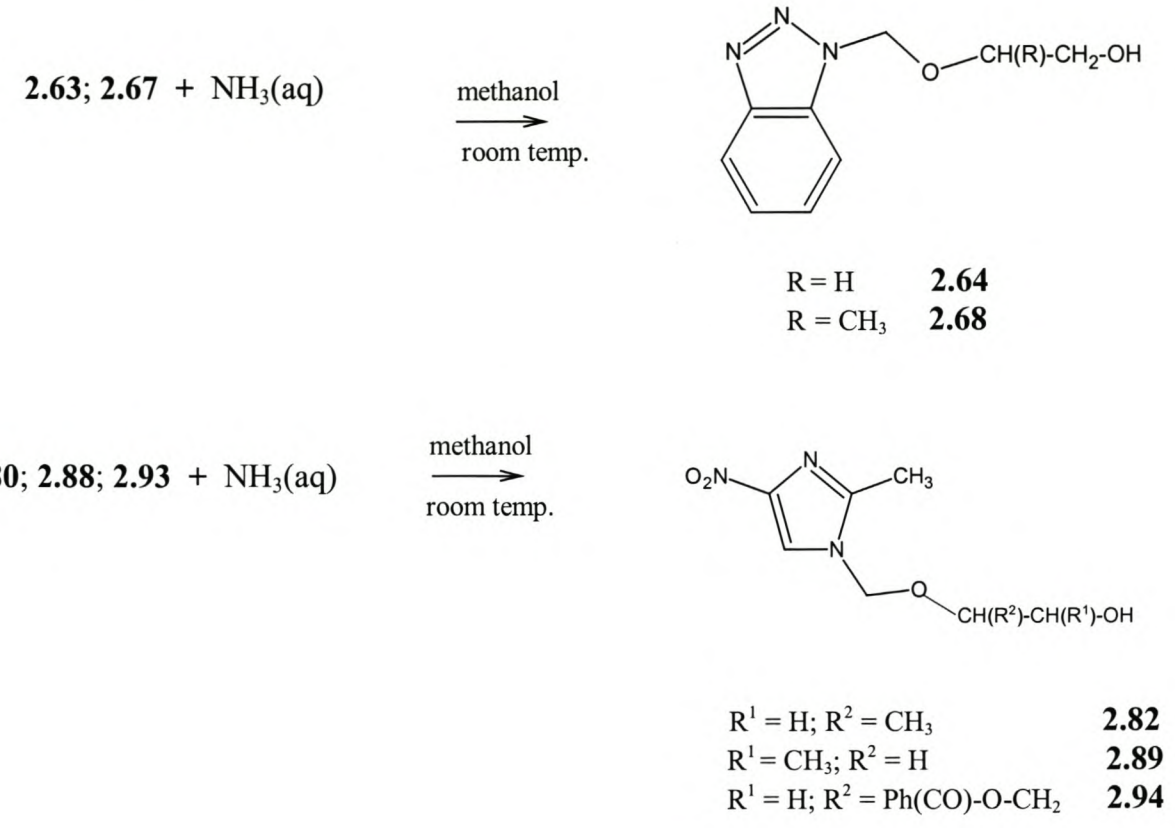
2.3.2.1 Preparation of *N*-alkylated intermediates for the synthesis of iodination precursors containing a leaving group in a β-position with respect to an oxygen atom

As a rule-of-thumb, the terms "β-ester" and "β-hydroxy" refer to the position of the ester group or the hydroxy group (β) relative to the ether oxygen atom in the prosthetic group. Precursors **2.65**, **2.69**, **2.83**, **2.85**, **2.90**, **2.95** and **2.97** all fall in the category mentioned in the heading. The respective intermediates were prepared in a two-step procedure involving the *N*-alkylation of the heterocyclic nitrogen compounds as the first step. For this, the specially prepared chloromethyl ethers were used. The *N*-alkylation step was followed by hydrolysis of the protecting ester function to obtain the target β-hydroxy intermediates. The reaction schemes in Scheme 2.13 illustrate these *N*-alkylation reactions and give the β-esters that were formed in the first step of these syntheses.



Scheme 2.13 Synthesis of the *N*-alkylated benzotriazole and 2-methyl-4-nitroimidazole derivatives

These β-esters were subsequently hydrolysed according to the reaction schemes in Scheme 2.14 to give the β-hydroxy compounds **2.64**, **2.68**, **2.82**, **2.89** and **2.94**. These alcohols were the intermediates for the synthesis of the precursors.



Scheme 2.14 Synthesis of the *N*-alkylated benzotriazole and 2-methyl-4-nitroimidazole β-hydroxy compounds

The preparation of the various condensation products, as outlined in Scheme 2.13, as well as their hydrolysis products, as outlined in Scheme 2.14, will now be discussed in greater detail.

2.3.2.1.1 Preparation of the condensation products **2.63**, **2.67**, **2.80**, **2.88**, **2.93**

Because of the instability of the chloromethyl ethers (*Guédin-Vuong & Nakatani, 1986*), it was decided to use the milder reaction conditions described by Waterhouse *et al.* (1996) for the preparation of these condensation products. The amine and a three-molar excess of anhydrous potassium carbonate were added to dichloromethane (or dried acetonitrile in the case of 2-

methyl-5-nitroimidazole), followed by the addition of a slight molar excess (based on the mono/di-ester starting material content) of the chloromethyl ether in dichloromethane or acetonitrile. The mixture was stirred at room temperature for a few hours until TLC (Methods 6, 7 or 8) showed partial or total consumption of the amine. According to HPLC [Method 2(b)], the condensation of benzotriazole and 2-chloromethoxyethyl benzoate **2.51** gave a major product (64%) (1-[(2-benzoyloxyethoxy)methyl]benzotriazole **2.63**), unreacted benzotriazole, as well as unknown by-products. These by-products could possibly have been formed as a result of alkylation of the benzotriazole anion *N*-2, but this was not verified. The isolated crude product was purified, using column chromatography over silica gel, to give a fraction with a purity of 94% in a yield of 24%, as well as a less pure fraction with a purity of 85% in a yield of 35%.

According to HPLC [Method 2(b)], the condensation of benzotriazole and the isomeric mixture of 2-chloromethoxyprop-1-yl benzoate **2.56** and 1-chloromethoxyprop-2-yl benzoate **2.57** (resulting from impure 1-bromo-2-propanol **2.52** and therefore impure 2-hydroxyprop-1-yl benzoate **2.54**, as shown in Scheme 2.7) gave two major components with retention times differing by approximately 0.5 min. in a ratio of 79:21. Together, they constituted approximately 65% of all the constituents of the product. These two compounds were the isomeric condensation products resulting from the isomeric mixture of **2.56** and **2.57**. The major condensation product, formed in a concentration of 79%, was 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67**. Compound **2.67** and its isomer could not be completely separated by means of column chromatography over silica gel. The major product (**2.67**) was nevertheless purified to an isomeric purity of 76% by this method. Attempts to crystallise the purified product **2.67** failed.

HPLC analysis of the product (**2.67**) formed in the alkylation of benzotriazole with pure 2-chloromethoxyprop-1-yl benzoate **2.56**, showed an isomeric purity of 97%. This high isomeric purity of **2.67** was to be expected, and it reflects the 97% isomeric purity of 2-hydroxyprop-1-yl benzoate **2.54**, the starting material of the alkylating agent **2.56**. 1-[(1-Benzoyloxyprop-2-

yloxy)methyl]benzotriazole **2.67**, prepared from pure **2.56**, was crystallisable from methanol after silica gel purification of the crude reaction product. The crystalline product had an overall purity of 99%, without any isomeric product.

The synthesis of 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80** was carried out only using the isomeric mixture of 2-chloromethoxyprop-1-yl benzoate **2.56** and 1-chloromethoxyprop-2-yl benzoate **2.57** as alkylating agent. This was done before the purer **2.56** became available. According to HPLC analysis (Method 5), the condensation of 2-methyl-5-nitroimidazole and impure **2.56** resulted in a product with relatively low purity (approximately 37%). TLC (Method 9) of the crude product showed the presence of two components with very similar R_f's. Column chromatography over silica gel, using ethyl acetate as mobile phase, gave a first collected fraction enriched with respect to the less polar of these components. The HPLC retention time of this fraction corresponded to that of the 37% peak shown in the chromatogram of the crude reaction product. In contrast to the benzotriazole condensation product 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67** prepared from the impure **2.56,** this semi-pure nitroimidazole product was crystallisable from methanol. Upon crystallisation, a solid white product was obtained in a total yield of approximately 33%, and in a purity of 98%. NMR and MS proved this product to be the condensation product **2.80**. This result shows that a purer alkylating agent is not necessarily needed to obtain a pure nitroimidazole condensation product, unless a higher yield of product is required. A second collected fraction, enriched with respect to the more polar component, could not be crystallised. The chemical shift values of the heterocyclic ring carbons in the ^{13}C NMR spectrum of the compound present in this fraction were in good agreement with those of authentic 2-methyl-5-nitroimidazole derivatives (see Table 2.3). This compound is therefore 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-5-nitroimidazole **2.81**.

The condensation product 1-[(2-benzoyloxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.88**, prepared from 2-methyl-5-nitroimidazole **2.79** and 1-chloromethoxyprop-2-yl benzoate **2.57**, had an isomeric purity of only 74%, as shown by HPLC (Method 4). This was to be

expected, as the isomeric purity of the monoester 1-hydroxyprop-2-yl benzoate **2.55**, the starting material in the synthesis of **2.57**, was only 70% (see Section 2.3.1.3). The crude product was chromatographed over silica gel in order to remove most of the impurities and this was followed by crystallisation of most of the contaminating isomer, 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80**, from a methanol solution of the purified **2.88**. The mother liquor containing the enriched **2.88** was once again chromatographed over silica gel to obtain **2.88** with an isomeric purity of 90%. The purified product was isolated in a yield of 31%.

As mentioned in Section 2.3.1.4, the synthesis of 1-[(1,3-dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.93** by condensation of the heterocyclic amine, 2-methyl-5-nitroimidazole **2.79**, with the alkylating agent 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61**, was not as successful as the condensation with the other agents, 2-chloromethoxyethyl benzoate **2.51**, 2-chloromethoxyprop-1-yl benzoate **2.56** and 1-chloromethoxyprop-2-yl benzoate **2.57**. This was blamed on the low and varying purity of **2.61**, prepared from 1,3-dibenzoyloxy-2-propanol **2.59**. HPLC chromatograms (Method 5) always showed some unreacted starting material, varying amounts of 1,3-dibenzoyloxy-2-propanol **2.59** (indicating its poor conversion to chloromethyl ether **2.61**), as well as two new peaks with slightly different retention times with respect to each other. The ratio in which the desired product was formed in relation to all the other constituents varied substantially from 6 to 33%, but the ratio in which the two major constituents was formed was always in the order of 60:40. The identity of the major product, which was the less polar component on TLC, was confirmed by ^{1}H and ^{13}C NMR to be 1-[(1,3-dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.93**. The chemical shift values of the heterocyclic ring carbons in the ^{13}C NMR spectrum of the minor, more polar fraction were in good agreement with those of authentic 2-methyl-5-nitroimidazole derivatives (see Table 2.3), indicating it to be the 2-methyl-5-nitroimidazole analogue of **2.93**. Despite the highly impure reaction products, purification with silica gel chromatography proved to be fairly successful. The reaction in which the major product

constituted 33% of all the constituents by HPLC gave a yield of approximately 28% in a purity of 96%.

2.3.2.1.2 Preparation of the β-hydroxy intermediates **2.64**, **2.68**, **2.82**, **2.89**, **2.94**

The next step in the synthesis of the intermediates required for the synthesis of the precursors was to convert the condensation products to the β-hydroxy intermediates by alkaline hydrolysis, as outlined in Scheme 2.14. Hydrolysis of the various condensation products was carried out in methanolic ammonia according to the method used by Srivastava *et al.* (1991). The hydrolysis products were extracted from the aqueous reaction mixtures with chloroform, toluene or diethyl ether. Chloroform and ether proved to be the most efficient extraction solvents. The hydrolysis of 1-[(2-benzoyloxyethoxy)methyl]benzotriazole **2.63** gave a product that contained one major constituent, namely 1-[(2-hydroxyethoxy)methyl]benzotriazole **2.64** (HPLC, Method 2b). The chloroform extract of this product was not purified but used directly for the synthesis of the corresponding tosylate precursor. The HPLC chromatogram (Method 2b) of 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68**, prepared from 76% isomerically pure 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67**, also showed a single peak. However, when changing the HPLC conditions (Method 6), resulting in an increase of the retention time from 5 to 16 minutes, a more polar constituent with a slightly shorter retention time appeared as a shoulder to the peak of the major constituent. The difference in retention times was approximately 0.2 minutes and the ratio of the minor to the major peak was 20:80. This shoulder was absent when compound **2.68** was prepared from 99% pure **2.67**. These results suggest that the shoulder can probably be ascribed to the presence of the other isomer in the product. Compound **2.68**, prepared from 99% pure **2.67**, was chromatographed over silica gel to give a main fraction in a yield of 91%.

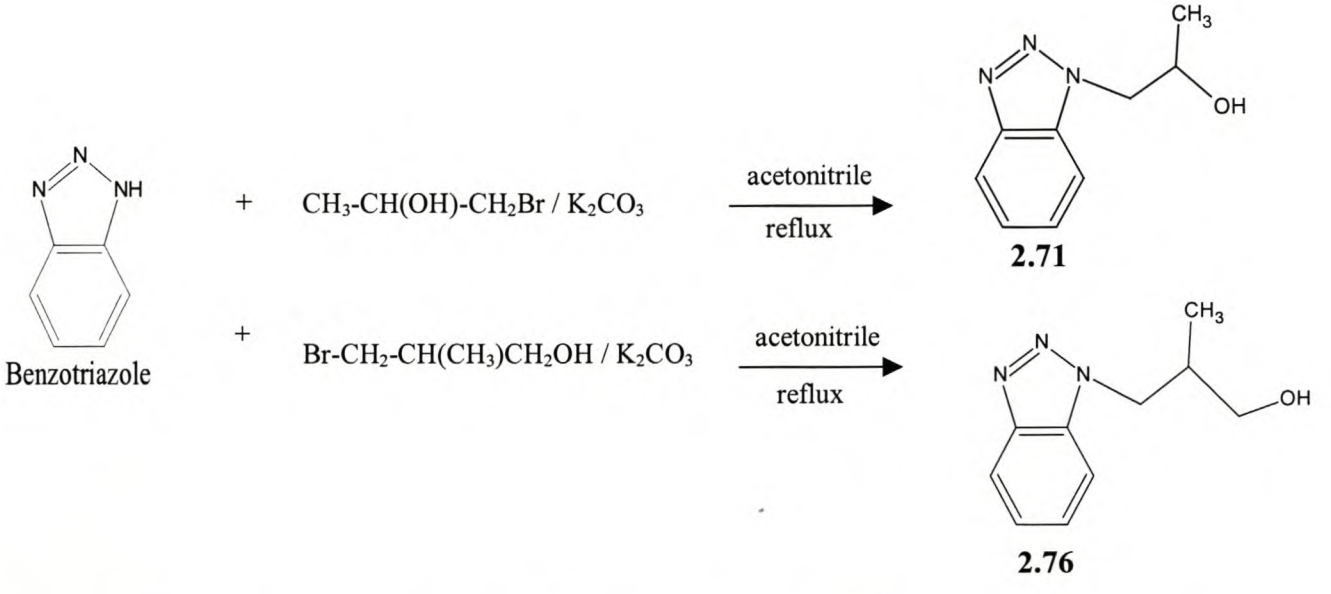
As expected, the high purity of 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80** resulted in an almost completely pure hydrolysis product 1-[(1-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.82** (HPLC, Method 6). After

purification, **2.82** was obtained in a yield of 93-97%. The yield of the hydrolysis product 1-[(2-hydroxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.89** after silica gel chromatography was 90%, and the 89% isomeric purity of **2.89** relates well to the 90% purity of its starting material 1-[(2-benzoyloxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.88**. The isomeric purity of **2.89** was increased to 94% by crystallisation from ethyl acetate, but resulted in a reduced overall yield of 55%.

For the conversion of the condensation product 1-[(1,3-dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.93** to a suitable precursor for labelling, two approaches were possible. In the one approach, both ester groups in **2.93** could be hydrolysed, resulting in a nucleoside analogue similar to compound **1.31**. One of the hydroxyl groups in this analogue could then be protected by selective acetylation, followed by the conversion of the other one to a suitable leaving group according to the method of Wada *et al.* (2000). Another approach would be to hydrolyse only one of the benzoyloxy groups of **2.93**, resulting in a nucleoside with one of the hydroxyl groups already protected, and thereby eliminating the need to protect one of the hydroxyl groups again, as required in the first approach. The second approach was selected for obvious reasons. The diester **2.93** was therefore partially hydrolysed in methanolic ammonia to give the hydroxyester 1-[(1-benzoyloxy-3-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.94**. The reaction was carefully monitored by means of HPLC (Method 5), which showed the formation of **2.94**, as well as a small amount of the dihydroxy compound, the formation of which could be suppressed by decreasing the ammonia content of the reaction mixture to a molar ratio of approximately 48 mmoles NH_3 per mmole **2.93**. A molar ratio of 190-370 mmoles NH_3 per mmole ester was used in the hydrolyses of the other condensation products. Quantitative and selective isolation of the hydroxyester **2.94** was achieved by extracting the water-diluted reaction mixture with chloroform. All the polar impurities, including the dihydroxy compound, remained in the aqueous phase. The crude extract was chromatographed over silica gel, giving 96% pure **2.94** in a yield of 57%.

2.3.2.2 Preparation of *N*-alkylated intermediates for the synthesis of precursors lacking a β-oxygen atom

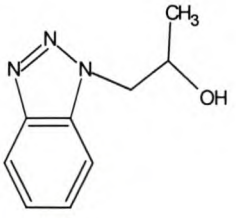
The benzotriazole-derived precursors 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73** and 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.77** fall in this category. The rationale behind the selection of these compounds as precursors is explained in paragraph 2.3. The synthesis of their respective *N*-alkylated intermediates 1-(2-hydroxyprop-1-yl)benzotriazole **2.71** and 1-(3-hydroxy-2-methylprop-1-yl)benzotriazole **2.76** was much more straightforward than the synthesis of the intermediates required for the preparation of the precursors containing a β-oxygen atom. The *N*-alkylated intermediates **2.71** and **2.76** were synthesised according to the method used by Yasunaga *et al.* (1998), as illustrated in Scheme 2.15.



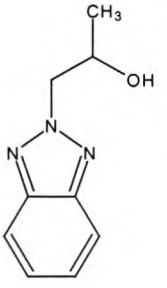
Scheme 2.15 Synthesis of the *N*-alkylated benzotriazole intermediate compounds used for the preparation of precursors lacking a β-oxygen atom.

The alkylating agent used to prepare 1-(2-hydroxyprop-1-yl)benzotriazole **2.71** was the isomerically purer 1-bromo-2-propanol **2.52**, prepared according to Scheme 2.10. The reaction mixture was heated under reflux. HPLC analysis of the reaction mixture (Method 7) revealed

the presence two products eluting about six minutes apart, in a ratio of 72:28. The more polar compound, with the shorter retention time, was the major component. The two components were separated by column chromatography over silica gel. The more polar component was obtained in a 47% yield and in a purity of 97%. According to the data in Table 2.4, the ^{13}C NMR spectrum of this component had six signals in the aromatic region of the spectrum, thereby confirming it to be 1-(2-hydroxyprop-1-yl)benzotriazole **2.71**, the product of alkylation at the *N*-1 atom in the heterocyclic ring. The ^{13}C NMR spectrum of the less polar component only had three signals in its aromatic region which can be ascribed to the presence of three pairs of equivalent aromatic carbons. This component was therefore assumed to be 2-(2-hydroxyprop-1-yl)benzotriazole **2.72**, resulting from alkylation at *N*-2 (see Scheme 2.12).



2.71



2.72

TABLE 2.4 Comparison of ^{13}C NMR data of compounds **2.71** and **2.72**

Compound	^{13}C NMR chemical shift values of the aromatic ring carbons (ppm)
2.71	109.93, 119.45, 123.96, 127.34, 133.69, 145.35
2.72	118.02, 126.73, 144.35

The reaction conditions used for the synthesis of 1-(2-hydroxyprop-1-yl)benzotriazole **2.71** were also used successfully for the synthesis of 1-(3-hydroxy-2-methylprop-1-yl)benzotriazole **2.76**, which was required as starting material for the preparation of 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.77**. Benzotriazole was coupled to the

bromohydrin (*S*)-(+)-3-bromo-2-methyl-1-propanol **2.75** as shown in Scheme 2.15. HPLC analysis of the resulting product mixture (Method 7) again revealed the presence of two products with retention times about nine minutes apart and in a ratio of 63:37. It was assumed that the minor product was formed by alkylation at *N*-2, but this was not confirmed. The two compounds were separated by means of silica gel chromatography to produce pure 1-(3-hydroxy-2-methylprop-1-yl)benzotriazole **2.76** in 44% yield. It is interesting to note that the *N*-alkylations with this reagent, as well as with 1-bromo-2-propanol, both required elevated reaction temperatures compared to the *N*-alkylations with the chloromethylethers 2-chloromethoxyethyl benzoate **2.51** and 2-chloromethoxyprop-1-yl benzoate **2.56**, which were carried out at room temperature. The condensation reaction with the bromohydrin 3-bromo-2-methyl-1-propanol at room temperature, for example, yielded only 7% product, 5% by-product and 86% unreacted benzotriazole after 3.5 hours, illustrating the low reactivity of the halohydrins as compared to the chloromethylethers.

2.3.3 Preparation of the Amine Precursors

The last step in the preparation of the precursors was the conversion of the hydroxyl group of the *N*-alkylated intermediates to a tosylate or a triflate group. The tosylate group was generally preferred as leaving group because of the superior stability of tosylate compounds as opposed to triflates. The tosylates could therefore be stored for longer periods without significant decomposition. The tosylates were generally prepared according to the method used by Kiesewetter *et al.* (1986). The reactions were carried out in dichloromethane, with *p*-toluenesulphonyl chloride (*p*-TsCl) as tosylating agent and triethylamine as base. The reaction mixtures were heated under reflux until all or most of the starting material had been consumed as monitored by TLC (Methods 7 or 8). In two cases, the tosylation reaction was carried out in pyridine according to the method used by Argentini *et al* (1981). This method is useful for small-scale reactions because the reaction is carried out without heating. However, due to the more cumbersome work-up procedure, the Kiesewetter's method was generally preferred. As explained earlier in paragraph 2.3, 2-methyl-4-nitro-1-[(2-trifluoromethanesulphonyloxyprop-

1-yloxy)methyl]imidazole **2.90** was prepared as the triflate rather than the tosylate. Another triflate, 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85**, was also prepared, in addition to its tosylate analogue 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.83**. The triflates were prepared according to the method used by Jerabek *et al.* (1986). Crude tosylated products were purified by column chromatography over silica gel in order to separate unreacted starting material, *p*-TsCl and other by-products from the target compound. The triflates were not purified, as the conversions appeared to have proceeded quantitatively (TLC). They were also not characterized because of their instability in a solid form (*Jerabek et al., 1986*).

2.3.3.1 Preparation of iodination precursor 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65**

Starting from the β-hydroxy intermediate 1-[(2-hydroxyethoxy)methyl]benzotriazole **2.64**, the compound was initially prepared according to the method used by Kiesewetter *et al.* (1986). Column chromatography over silica gel assumedly gave the tosylate **2.65** in what was calculated to be 34% yield. Its ^{1}H NMR spectrum, however, did not contain the characteristic methyl and aromatic protons of the tosyl group. According to the data shown in Table 2.5, the aliphatic region of the spectrum also showed certain similarities to those of the iodinated product 1-[(2-iodoethoxy)methyl]benzotriazole **2.66b**, prepared from an authentic precursor **2.65**. Only the chemical shifts of the respective terminal methylene protons differed. This suggested that the terminal methylene protons of the two compounds were attached to two different atoms with different electronegativities. This evidence suggested that this fraction was not the tosylate but the corresponding chlorinated product 1-[(2-chloroethoxy)methyl]benzotriazole **2.66a**. Mass spectra data supported this assumption by clearly showing the two molecular ions of compound **2.66a**, arising from the two stable chlorine isotopes ^{35}Cl and ^{37}Cl [*m/z* 211(27%); 213 (8%)], in the correct isotopic abundance ratio. In addition to this all, the reactivity of **2.66a** towards radioiodide was also much lower than would be expected for the tosylate (see Section 3.4.1). According to Kiesewetter *et al.*

(1986), chlorination of the highly reactive tosylates is possible in the presence of chlorinated solvents. Compound **2.66a** was formed in a significant amount during the course of the reaction. The tosylation reaction was repeated, using pyridine as solvent according to the method used Argentini *et al.* (1981). Purification gave the target tosylate **2.65** in a yield of 62%. Radiosynthesis of 1-[(2-[^{123}I]iodoethoxy)methyl]benzotriazole **2.66b** was initially carried out by using 1-[(2-chloroethoxy)methyl]benzotriazole **2.66a** as the precursor, although 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65** was also used later.

TABLE 2.5 Comparison of ^{1}H NMR (300 MHz) data of the aliphatic protons of 1-[(2-chloroethoxy)methyl]benzotriazole **2.66a** and 1-[(2-iodoethoxy)methyl] benzotriazole **2.66b**

Compound **2.66a**			Compound **2.66b**		
Protons	δ (ppm)	J (Hz)	Protons	δ (ppm)	J (Hz)
OCH_2CH_2Cl	3.55-3.82 (4H) m	-	CH_2I	3.08-3.12 (2H) t	6.5
			OCH_2	3.71-3.75 (2H) t	6.5
NCH_2	6.12 (2H) s	-	NCH_2	6.03 (2H) s	-
Ts-CH_3	no signal				

2.3.3.2 Preparation of iodination precursor 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)-methyl]benzotriazole **2.69**

The tosylated benzotriazole precursor **2.69** was prepared from both the 80% and 100% isomerically pure 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68** (see Section 2.3.2.1.2). The reaction was carried out according to the dichloromethane/triethylamine method used by Kiesewetter *et al.* (1986). The reaction mixtures were heated by refluxing either on a

hot plate or with the reaction vessel immersed in an oil bath[6] at 120°C. The reaction product was purified by column chromatography over silica gel. A higher yield of the product (91%) was obtained by heating the reaction mixture in an oil bath at 120°C, compared to a yield of 36% obtained when the reaction vessel was suspended above a hot plate. HPLC analysis [Method 2(b)] showed that the purity of the tosylated benzotriazole **2.69** obtained from the 80% pure 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68** was 93%, while the 100% pure **2.68** gave **2.69** in a purity of 98%. Apparently the isomeric impurity [Ar-N-CH_2-O-CH_2-$CH(CH_3)$-OH] in the 80% pure **2.68** was not tosylated quantitatively, presumably because of the steric hindrance resulting from the fact that its hydroxyl group is attached to a secondary carbon. This implies that 1-bromo-2-propanol does not need to be 100% pure in order to obtain a relatively pure tosylate.

2.3.3.3 Preparation of iodination precursor 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73**

Using the intermediate 1-(2-hydroxyprop-1-yl)benzotriazole **2.71** as the starting material, the reaction was carried out in dichloromethane/triethylamine at an oil bath temperature[6] of 140-160°C. A feature of this reaction was the formation of a by-product during prolonged heating in an unsuccessful attempt to force the reaction to completion. After seven hours at 140-160°C, the ratio of product:by-product was approximately 1:1 according to HPLC analysis [Method 2(b)]. The unidentified by-product was not isolated and characterized. Column chromatography over silica gel gave a fraction with a purity of 92% in a yield of 37%. Crystallisation of this material from ethyl acetate gave the target compound **2.73** in a purity of 97%. ^{1}H NMR analysis confirmed the presence of the tosylate group in the product.

[6] The temperature of the oil bath only indicates the surrounding temperature of the reaction mixture, and not the temperature of the reaction mixture as such

2.3.3.4 Preparation of iodination precursor 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)-benzotriazole **2.77**

The reaction was carried out in dichloromethane at an oil bath temperature of 140°C, using the intermediate 1-(3-hydroxy-2-methylprop-1-yl)benzotriazole **2.76** as the starting material. The crude product was chromatographed twice over silica gel to give the target compound **2.77** in a yield of 50% and a purity of 92%.

2.3.3.5 Preparation of iodination precursor 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxy-prop-2-yloxy)methyl]imidazole **2.83**

The reaction was carried out in dichloromethane for 4.5 hours at an oil bath temperature of 100°C, using the highly pure β-hydroxy intermediate 1-[(1-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.82** as the starting material. The conversion proceeded smoothly (TLC, Method 10), giving a 96% pure target compound **2.83** in a yield of 91%, after chromatography over silica gel. This yield corresponds well to that obtained in the synthesis of the benzotriazole analogue 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69** at 120°C (see Section 2.3.3.2). This was to be expected as both compounds have identical prosthetic groups and the reaction conditions were similar.

2.3.3.6 Preparation of iodination precursors 2-methyl-4-nitro-1-[(1-trifluoromethanesulpho-nyloxyprop-2-yloxy)methyl]imidazole **2.85** and 2-methyl-4-nitro-1-[(2-trifluoro-methanesulphonyloxyprop-1-yloxy)methyl]imidazole **2.90**

These two triflate precursors were prepared from the β-hydroxy intermediates 1-[(1-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.82** and 1-[(2-hydroxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.89** respectively. They were prepared at the same time in order to monitor their respective stabilities versus time. The triflates were prepared by treating the β-hydroxy compounds with trifluoromethanesulphonic (triflic) anhydride at -10°C

in a solution of dry acetonitrile. Extraction with dichloromethane from an aqueous mixture, followed by evaporation to dryness, gave the products in quantitative yields. Triflate **2.85** was obtained as a white solid and showed a single spot on TLC (Method 11). Triflate **2.90** was obtained as an oil and showed a few minor impurities on the same TLC system. Both products were stored as solutions in dichloromethane at -10°C. Triflate **2.85** appeared to be fairly stable in a dichloromethane solution. No significant decomposition occurred over a period of approximately three months, as judged by TLC. The triflate **2.90**, however, decomposed almost completely over the same period.

2.3.3.7 Preparation of iodination precursor 1-[(1-benzoyloxy-3-trifluoromethanesulphonyl-oxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.95**

Iodination precursor **2.95** was prepared from 1-[(1-benzoyloxy-3-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.94** in a way similar to the preparation of the other triflates **2.85** and **2.90**. TLC showed an almost complete conversion of the starting material **2.94** to the target compound **2.95**. However, after work-up of the reaction mixture according to the procedure followed for the isolation of **2.85** and **2.90**, HPLC analysis [Method 2(b)] unexpectedly showed the almost complete decomposition of the triflate **2.95**, with the simultaneous reappearance of the starting material **2.94**. It was not clear why the triflate **2.95** was unstable during aqueous work-up, whereas **2.85** and **2.90** stayed intact. It was subsequently decided not to use the aqueous work-up procedure for **2.95**, but to use this triflate *in situ* for iodinations and radioiodinations.

2.3.3.8 Preparation of iodination precursor 1-[(1-benzoyloxy-3-*p*-toluenesulphonyloxy-prop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.97**

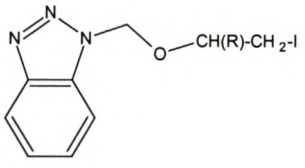
Because of the instability of the triflate **2.95**, as well as the low radiochemical yields obtained using the triflate that had been prepared *in situ*, it was decided to also prepare its tosylated analogue **2.97** from compound **2.94**. This synthesis was done according to the method of Wada

et al. (2000), in which the free hydroxyl group in the mono-acetyl ester of 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole **1.31** was converted into a tosylate. This method is essentially similar to that used by Argentini *et al.* (1981), in which pyridine is used as solvent. TLC (Method 9) showed an almost complete conversion of the starting material into the product. After removal of the pyridine and purification by column chromatography over silica gel, the pure target tosylated iodination precursor **2.97** was obtained in a yield of 79%.

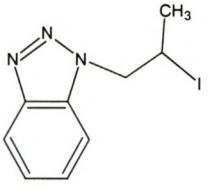
2.3.4 Preparation of the Iodinated Heterocyclic Amine Derivatives

In order to obtain "cold" iodinated compounds for characterisation purposes (NMR, MS), portions of the precursors were converted into their respective iodinated analogues. In most cases these iodo compounds were prepared from their respective tosylate precursors, except for 1-[(2-iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92** and 1-[(1-benzoyloxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.99**, which were prepared from the respective triflate precursors. In the case of the tosylates, the precursor was heated in an acetone solution in the presence of a slight molar excess of sodium iodide. In the case of the triflates, the crude triflate was reacted with sodium iodide at room temperature after replacing the acetonitrile, in which the triflate had been formed, with acetone. The conversions were complete within 15-20 minutes. Sodium triflate and traces of triflic acid were removed from the crude reaction mixtures by extracting a dichloromethane or chloroform solution of the crude product with aqueous sodium carbonate or bicarbonate solution and/or filtering it through a column of aluminium oxide. Compound **2.99** was converted to 1-[(1-hydroxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100** by means of alkaline hydrolysis with sodium hydroxide. All the crude reaction products were chromatographed on silica gel to give the purified iodinated compounds 1-[(2-iodoethoxy)methyl]benzotriazole **2.66b**, 1-[(1-iodoprop-2-yloxy)methyl]benzotriazole **2.70**, 1-(2-iodoprop-1-yl)benzotriazole **2.74**, 1-(3-iodo-2-methylprop-1-yl)benzotriazole **2.78**, 1-[(1-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87**, 1-[(2-iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92**, 1-[(1-

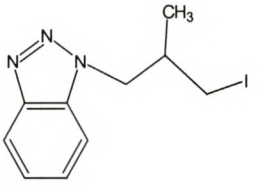
benzoyloxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.99** and 1-[(1-hydroxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100**.



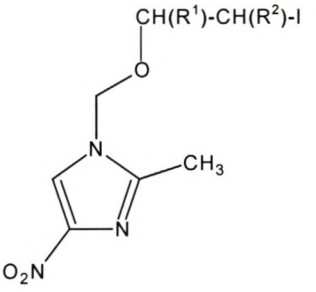
R = H **2.66b**
R = CH_3 **2.70**



2.74

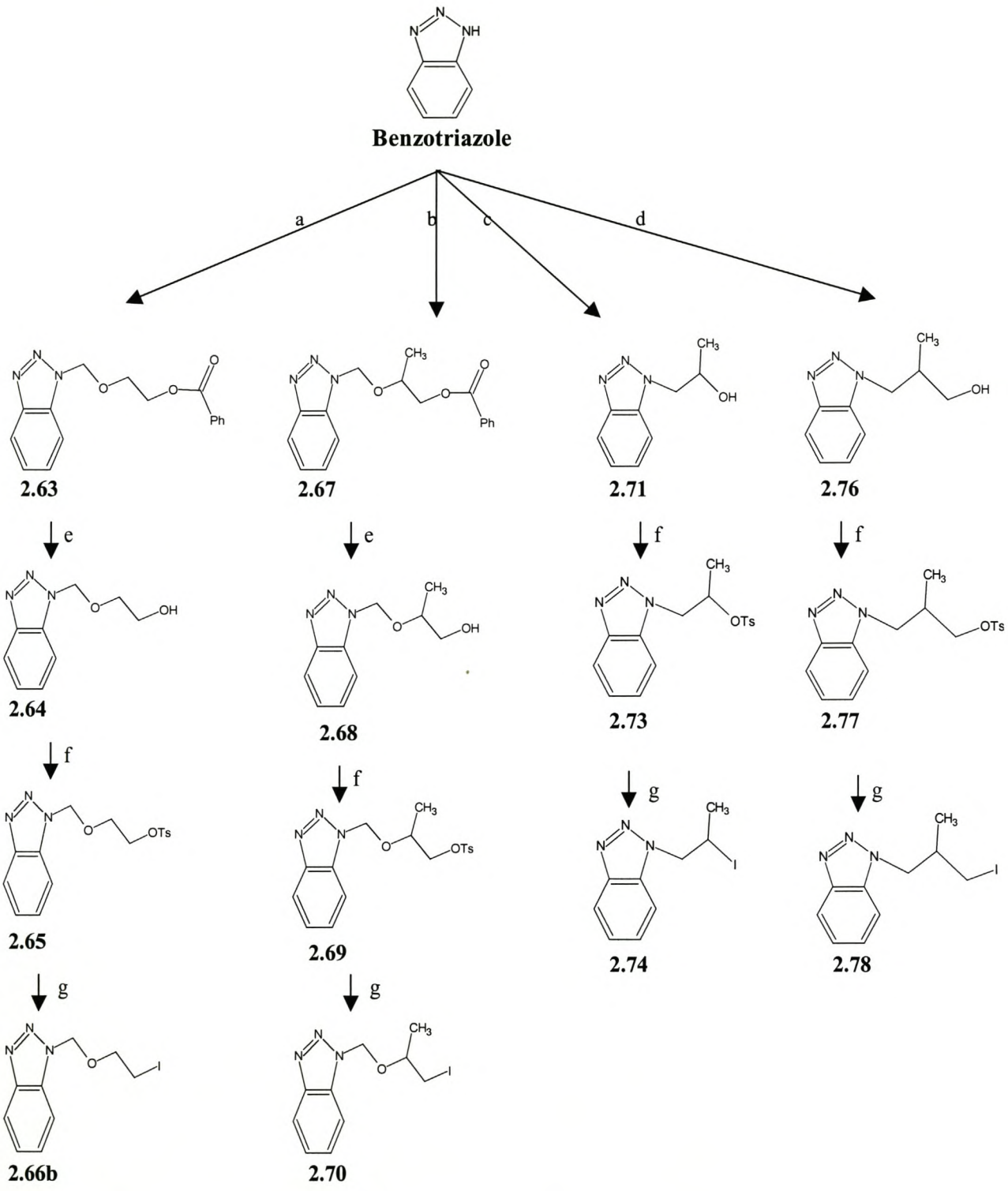


2.78



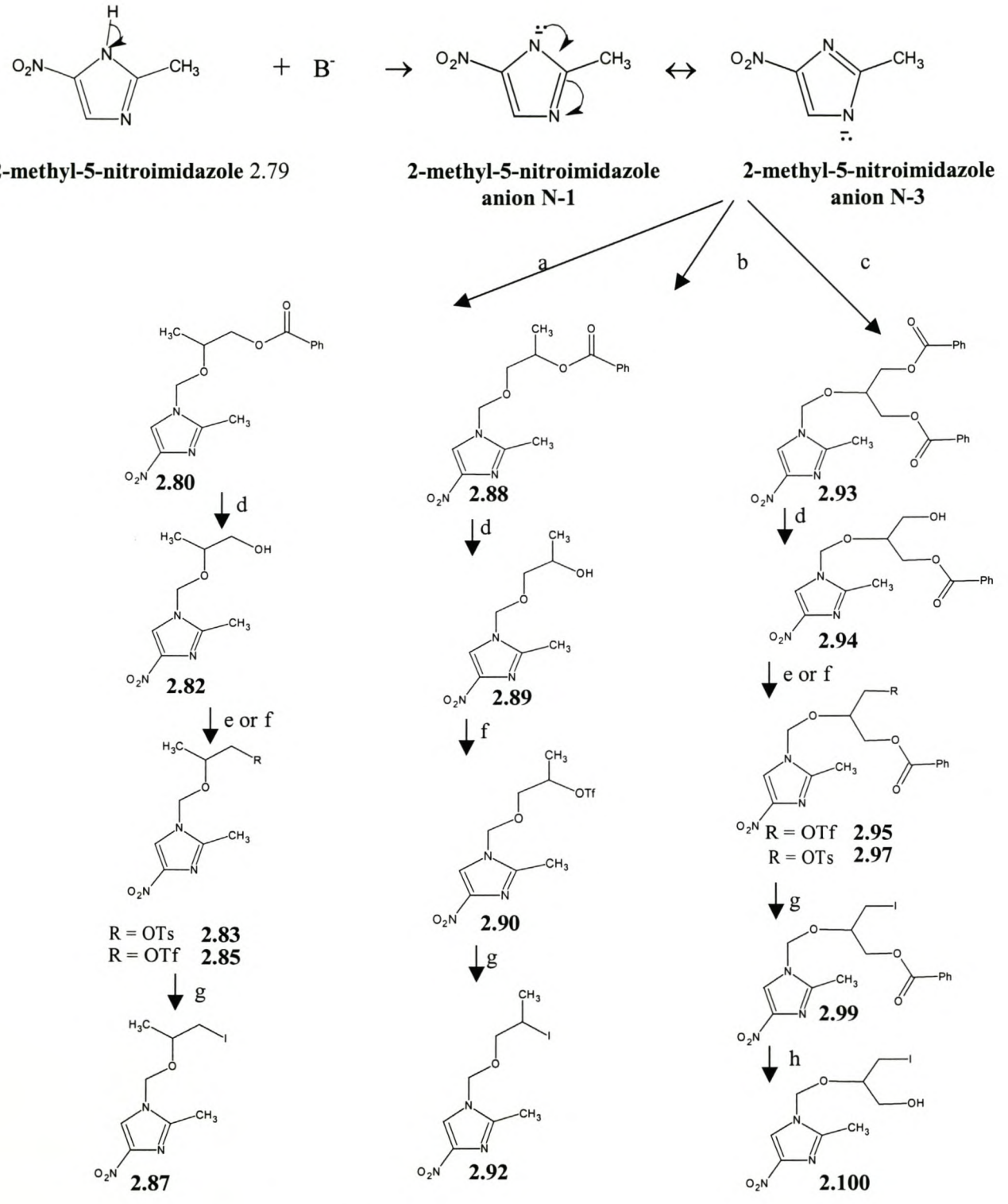
$R^1 = CH_3$; $R^2 = H$	**2.87**
$R^1 = H$; $R^2 = CH_3$	**2.92**
$R^1 = PhCOO\text{-}CH_2$; $R^2 = H$	**2.99**
$R^1 = HO\text{-}CH_2$; $R^2 = H$	**2.100**

The syntheses of the *N*-alkylated benzotriazole and 2-methyl-4-nitroimidazole derivatives, the various iodination precursors and the iodinated compounds are summarized in Schemes 2.16 and 2.17.



a. 2-chloromethoxyethyl benzoate **2.51** b. 2-chloromethoxyprop-1-yl benzoate **2.56**
c. 1-bromo-2-propanol **2.52** d. 3-bromo-2-methyl-1-propanol **2.75** e. NH_3 (aq) f. *p*-TsCl g. NaI

Scheme 2.16 Synthesis of benzotriazole derivatives, iodination precursors and iodinated compounds



a. 2-chloromethoxyprop-1-yl benzoate **2.56** b. 1-chloromethoxyprop-2-yl benzoate **2.57**
c. 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61** d. NH_3 (aq) e. *p*-TsCl f. triflic anhydride g. NaI
h. NaOH

Scheme 2.17 Synthesis of 2-methyl-4-nitroimidazole derivatives, iodination precursors and iodinated compounds

CHAPTER 3

RADIOLABELLING OF THE PRECURSORS WITH RADIOIODINE

3.1 GENERAL

In this study, two different radioisotopes of iodine, ^{123}I and ^{131}I, were used. While their physical properties such as atomic mass, half-life and mode of decay differ, their chemical properties are identical. The isotope with the shorter half-life, ^{123}I ($t_{1/2}$ = 13.2 hours), was used exclusively in the radiosynthesis of the benzamide and heterocyclic amine compounds, while the isotope with the longer half-life, ^{131}I ($t_{1/2}$ = 8.02 days), was used in the radiosynthesis of the model compounds during the pilot study. In the case of the radioiodinations carried out with ^{123}I, higher quantities of radioactivity were introduced into reaction mixtures to compensate for decay. Both these sources of radioiodine are carrier-free, that is, they contained no stable iodine. This implies that the radiolabelled products were also carrier-free, which means that they did not contain any of their cold iodinated analogues and therefore had high specific activities, as explained in Chapter 1. Furthermore, the radioiodinated species could generally be separated easily from the precursors by chromatographic means, which generally resulted in high effective specific activities.

Radioiodinations of the various tosylate and triflate precursors were generally done by iodide-for-tosylate/triflate exchange, while the tributylstannyl precursor **4.28** was radioiodinated by oxidative demetallation. Iodination with radioiodine or radioiodide was essentially similar to that involving the stable isotope, except for the difference in stoichiometry of the reactions. The ease with which the different radioiodinations proceeded, as well as the respective radiochemical yields, differed substantially. No deliberate attempts were made to optimise yields by systematic variation of the reaction parameters, as this was beyond the scope of this work. The synthesis of the radioiodinated model compounds is only discussed in general, whereas those of the benzamides and heterocyclic amines are discussed in greater detail.

Radiochemical yields are quoted, and in some cases yields of similar products are compared, for example when different types of precursors were used. The main object of this part of the work was to obtain sufficient amounts of the radiolabelled compounds for stability studies. Radioiodination reactions and stability studies were repeated at various stages of this investigation to test the reproducibility of the stability data. Radiolabelling of the tosylate and triflate precursors was generally carried out using the purest available precursor material.

Acetone or acetone/dioxane mixtures were used as solvents in the radioiodide-for-tosylate/triflate exchange reactions. Radioiodide is generally supplied as a solution in aqueous sodium hydroxide, which necessitated pre-drying of the solution, using a stream of nitrogen or argon before addition of the precursor in order to ensure anhydrous reaction conditions. Substitution reactions with the tosylates required heating of the reaction mixtures. Reaction mixtures were analysed by means of radio-HPLC and/or radio-TLC to determine the progress of a reaction, as well as to verify the authenticity of the major product. This was done by comparing the retention times of the major peaks in the radiochromatograms with those in the UV chromatograms of the corresponding cold iodinated reference compounds. A reversed phase C-18 column, the outlet of which was connected to a sodium iodide radiodetector coupled in series with a UV-detector, was used. Radiolabelling was always followed by purification by means of column chromatography over silica gel, which resulted in the separation of most of the unreacted radioiodide, as well as other radiochemical impurities, from the target compound. In most cases, this also resulted in the complete or partial separation of the labelled product from its lipophilic precursor, a requirement that was not essential for the purpose of this work. Radiochemical yields are expressed as the decay-corrected amount of activity obtained in a purified labelled form, as a percentage of the input activity in radioiodide form. HPLC analyses did not give an accurate reflection of the radiochemical yields, because, in some cases, substantial amounts of insoluble activity (up to 40% of the input activity) remained behind in the reaction vessels after the transfer of the reaction mixtures to the silica gel columns. It was not clear whether this residual activity was due to unreacted free

radioiodide or to partially precipitated radiolabelled products. Purified products were dissolved in human blood serum after removal of the organic solvent.

3.2 RADIOSYNTHESIS OF THE β-IODOALKYLETHER MODEL COMPOUNDS

The tosylates and dry radioiodide were heated in an acetone or acetone/dioxane mixture at high temperatures ranging from 120°C to 180°C. High temperatures were needed, presumably due to the inhibiting effect towards nucleophilic substitution (S_N) of an oxygen atom in a β-position relative to the leaving group (*Fleet, 1989*). Reactions were monitored chromatographically. Radiochemical yields (RCY) are displayed in Table 3.1.

TABLE 3.1 Radiochemical yields of ^{131}I-labelled β-iodoalkylethers

Compound	*Radiochemical Yield (%)
1-[^{131}I]iodo-2-phenoxyethane **2.4**	78 ± 10 (n=11)
2-benzyloxy-1-[^{131}I]iodoethane **2.7**	79 ± 15 (n=3)
1-[^{131}I]iodo-2-phenoxypropane **2.12**	77 ± 6 (n=3)
2-[^{131}I]iodo-1-phenoxy-propane **2.15**	66 ± 7 (n=5)
1-[^{131}I]iodo-2-phenoxy-butane **2.19**	72 ± 16 (n=3)
1-[^{131}I]iodo-2-methoxy-2-phenylethane **2.22**	60 ± 19 (n=6)

* Average decay-corrected yield, based on purified output activity, expressed as a percentage of total input activity

RCYs varied considerably, but were generally somewhat higher for the compounds containing straight-chain aliphatic groups ([^{131}I]**2.4** and [^{131}I]**2.7**). Compound [^{131}I]**2.15** was probably obtained in a lower RCY than compound [^{131}I]**2.12**, because the S_N reaction at a secondary carbon atom proceeded at a slower rate (*Fleet, 1989*). The generally lower and more

inconsistent RCYs obtained for [^{131}I]**2.22** can most probably be ascribed to the higher steric hindrance caused by the neighbouring phenyl group. Radio-TLC (Method 1) furthermore revealed the formation of some radiochemical impurities during the radiosynthesis of [^{131}I]**2.22**. These impurities, which were more polar than [^{131}I]**2.22**, tended to diminish or disappear completely on prolonged heating.

3.3 RADIOSYNTHESIS OF THE BENZAMIDE DERIVATIVES

These reactions, except for the synthesis of the iodoallyl product 2-(3-iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.48**, were all performed in acetone at reaction temperatures of 150° to 170°C for periods of up to 20 minutes. The reaction progress was monitored by means of radio-HPLC, generally using Method 1.

3.3.1 Radiosynthesis of 2-(2-[^{123}I]iodoethoxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.42**

Four experiments were conducted with the precursor 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)ethoxy]benzamide **2.41**. Radiochemical by-products were not formed in significant amounts. The retention times of the major peaks in the radiochromatograms were similar to those in the UV-chromatograms, confirming the authenticity of the radioiodinated compound. The radiochemical yields, according to HPLC, ranged from 72 to 94%, while the isolated yields after purification ranged from 65 to 83%. The purified fractions were completely free from the precursor.

3.3.2 Radiosynthesis of 2-(1-[^{123}I]iodoprop-2-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)-benzamide **2.44**

Three experiments were conducted with the precursor 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[1-(*p*-toluenesulphonyloxy)prop-2-yloxy]benzamide **2.43**. As in the previous case, the

reactions proceeded without the formation of significant amounts of radiochemical by-products. Comparisons of radioactivity and UV signals from the chromatographic analysis of [^{123}I]**2.44** and stable [^{127}I]**2.44** respectively, showed identical retention times. The isolated radiochemical yields were generally lower than those of [^{123}I]**2.42**, ranging from 57 to 67%. The purified product showed no trace of the precursor.

3.3.3 Radiosynthesis of 2-(2-[^{123}I]iodoprop-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)-benzamide **2.46**

Three experiments were conducted with the precursor 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)prop-1-yloxy]benzamide **2.45**. In contrast to its isomer, [^{123}I]**2.44**, the formation of this product was accompanied by the formation of varying amounts of radiochemical by-products. The HPLC purities ranged from 32 to 75%, while the total amount of impurities ranged from 10 to 57%. These impurities were more polar than the main product, and were unlikely to have arisen from impurities in the precursor **2.45**, which appeared to be fairly pure according to TLC (Methods 4 or 5). The formation of these impurities, which were not identified, resulted in radiochemical yields of the product varying between 32 and 65%.

HPLC analysis, using a C-18 column and isocratic elution with a mixture of 75% methanol and 25% water containing 0.1% triethylamine, gave a reasonable resolution of the two isomers [^{123}I]**2.44** and [^{123}I]**2.46**, with [^{123}I]**2.44** having the slightly shorter retention time (more polar). The radiochromatogram of [^{123}I]**2.46** had a small shoulder on the main peak, the retention time of which corresponded to that of [^{123}I]**2.44**. These retention times corresponded to those in the UV chromatograms of the non-radioactive analogues. The integration of this shoulder was obviously not very accurate, but [^{123}I]**2.44** constituted less than 10% of [^{123}I]**2.46**.

3.3.4 Radiosynthesis of 2-(3-[^{123}I]iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-yl-ethyl)benzamide **2.48**

The reaction conditions required for the radiosynthesis of [^{123}I]**2.48** differed from those employed in the radiosynthesis of the preceding radioiodinated compounds **2.42**, **2.44** and **2.46**, because of the different type of precursor, 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[3-(tributylstannyl)prop-2-en-1-yloxy]benzamide **2.47**, that was used. The labelling procedure was based on the method used by Musachio and Lever (1992) for the oxidative radioiododestannylation of *N*-alkylated spiperone analogues. Two experiments were conducted. The tributylstannylated precursor was reacted with ^{123}I at room temperature in the presence of chloramine-T as an oxidising agent. The work-up and purification procedure of the radioiodinated compound [^{123}I]**2.48** differed from Musachio and Lever's published method. After quenching the reaction and neutralisation of the reaction mixture, the organic component was extracted with dichloromethane and subsequently purified by chromatography over silica gel, similarly to the purification of [^{123}I]**2.42**, [^{123}I]**2.44** and [^{123}I]**2.46**. The two experiments gave an average overall radiochemical yield of 86% with radiochemical purities ranging from 94% to 99%. These figures illustrate the favourable properties of a tributyltin group as a leaving group for radioiodination. The purified product contained no detectable trace of the precursor. Its authenticity was confirmed by the corresponding retention times of the respective radio- and UV-signals.

3.4 RADIOSYNTHESIS OF THE HETEROCYCLIC AMINES

Radioiodinations of the tosylate precursors of the heterocyclic amine derivatives were carried out under reaction conditions similar to those used for the synthesis of the radioiodinated benzamide compounds. Slightly different reaction temperatures, ranging between 130° and 150°C, were used. Reaction times were in the order of 10 to 15 minutes. Radioiodinations of the triflate precursors were carried out at room temperature because of the high reactivity of the triflates. The progress of the reactions was monitored by radio-HPLC, using Method 2(a) or

2(b). It was found that substituting trifluoroacetic acid with triethylamine in the aqueous mobile phase did not result in any significant changes in retention times or peak resolution.

3.4.1 Radiosynthesis of 1-[(2-[^{123}I]iodoethoxy)methyl]benzotriazole **2.66b**

This radioiodinated compound was prepared from both the chlorinated precursor 1-[(2-chloroethoxy)methyl]benzotriazole **2.66a** (see Section 2.3.3.1) and the tosylated precursor 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65**. The chlorinated precursor **2.66a** gave yields of 32% and 51% at reaction temperatures of 130° and 150°C respectively, as determined by HPLC. At 150°C, a yield of 24% and a product purity of 93% were obtained after purification. The tosylated precursor **2.65** gave an average HPLC yield of 78% at a reaction temperature of either 130° or 150°C. In this case the temperature difference of 20°C did not appear to result in a significantly different yield. Yields obtained after purification varied between 48% and 70%, and purified fractions with an average radiochemical purity of 97% did not contain any detectable precursor. These figures confirmed the higher reactivity of tosylates in comparison to halogenated precursors, because the tosylate is known to be a much better leaving group. The radioiodinated products obtained from the two different precursors had corresponding retention times and these retention times also corresponded to that of the cold iodinated compound. The radioiodinations of both precursors were always accompanied by the formation of some radiochemical by-products that were less polar than the main product. The bulk of these impurities could be removed by means of column chromatography over silica gel.

3.4.2 Radiosynthesis of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]benzotriazole **2.70**

Four preparations were done with precursor 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69**. The retention time of the main product corresponded to that of the cold iodinated compound, thereby proving its authenticity. The yields and purities of the product varied considerably throughout all the experiments. The HPLC radiochromatogram of

a typical reaction mixture indicated the presence of a major impurity that appeared as a shoulder with a slightly longer retention time than the major product. Some other less polar minor impurities were also formed. On average [^{123}I]**2.70** represented 54% of all the radioactive species appearing in the chromatograms, including the unreacted radioiodide. The radiochemical purity appeared to deteriorate with the ageing of the precursor, despite its storage at -10°C. In one preparation, carried out approximately six months after the others, using the same precursor and identical reaction conditions, the ratio of the product versus the major impurity had changed from 84:16 to 69:31. Another more polar impurity, previously present only as a trace, now constituted 10% of the product. HPLC- [Method 2(a)] and TLC-analysis (Method 7) of the precursor **2.69** did not indicate any detectable decomposition of the precursor over time. Possible reasons for the increased formation of by-products were not investigated. As before, the majority of the impurities could be removed by column chromatography over silica gel. However, the major impurity could only be partially removed. Recovered yields of the purest fractions, having radiochemical purities between 90% and 95%, were in the order of 23%. These fractions also did not show detectable traces of the precursor.

3.4.3 Radiosynthesis of 1-(2-[^{123}I]iodoprop-1-yl)benzotriazole **2.74**

The precursor 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73** was used as starting material for the radiosynthesis of [^{123}I]**2.74**. The radiochemical yield of this compound was temperature dependent. At a reaction temperature that varied between 130° and 135°C, the recovered yield of the combined purified fractions was only in the order of 13-16%. At a temperature of 150°C, the purest fraction was isolated in yields varying between 31% and 42%, and its radiochemical purity varied between 95% and 98%. It showed no detectable trace of the precursor. The retention time of the main product peak corresponded to that of the UV-signal of the cold iodinated compound.

3.4.4 Radiosynthesis of 1-(3-[^{123}I]iodo-2-methylprop-1-yl)benzotriazole **2.78**

The precursor 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.77** was used as starting material for the radiosynthesis of [^{123}I]**2.78**. The reactions were carried out at either 130°C or 150°C. The yield at 130°C, as determined by HPLC, was in the order of 60% after 10 minutes. At 150°C, a highest yield of about 85% was obtained after 10 minutes. This once again demonstrated the temperature dependency of the substitution of tosylate with iodide. The retention time of the main product corresponded to that of the cold iodinated compound. As before, the good separation of the iodinated compound from its tosylated precursor resulted in a purified product free from any trace of the precursor. The recovered radiochemical yield obtained after the first reaction at 130°C was 38%, and the radiochemical purity of this fraction was approximately 98%. The second reaction at 150°C gave the product in a yield of 49% to 65%, and a purity of 97-99%.

3.4.5 Radiosynthesis of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87**

The tosylate 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.83** and the triflate 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85** were both used as precursor for the radiosynthesis of [^{123}I]**2.87.** The tosylate was only used in two preparations. For comparison, one of these experiments was conducted under the same conditions as were used in the experiment with the triflate as precursor. The results of this comparison will be discussed in paragraph 3.4.5.3.

3.4.5.1 Synthesis from tosylate **2.83**

The reaction with the tosylate **2.83** was carried out at 130°C and yielded a fairly pure product, practically free from other radiochemical impurities. This could be attributed to the fact that **2.83** was obtained in a high purity because one of the intermediates in its preparation, the condensation product 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80**,

was crystallisable. The yield, as determined by HPLC, was 58% and the balance was made up mainly by unreacted radioiodide. The retention time of the product peak corresponded to that of the UV-signal of the cold iodinated compound. The recovered radiochemical yield after purification was 47%, and the radiochemical purity was 95%. Unreacted radioiodide was the major impurity. The purified product contained a trace of the tosylate precursor because the two species could not be completely separated over silica gel. Various mobile phase combinations were tried, and the best separation was obtained with a mixture of ethyl acetate, hexane and chloroform (70:15:15). Even this system did not give complete separation of the tosylate and the iodinated product.

3.4.5.2 Synthesis from triflate **2.85**

The radioiodination reactions were initially carried out using the triflate **2.85** that had been purified by column chromatography over silica gel. The reaction was started at room temperature, and the reaction temperature was later increased to 65°C. After 75 minutes at room temperature, the yield, as determined by HPLC, was 42%. The reaction was not accelerated by increasing the temperature. When the radioiodinations were carried out using triflate **2.85** that had been purified by extraction with dichloromethane from an aqueous medium, a yield of 91%, as determined by HPLC, was obtained after 45 minutes at room temperature. A possible reason for this is that the silica gel-purified triflate probably still contained trifluoromethanesulphonic acid (a by-product from the preparation of the triflate). The acid could have inhibited the radioiodinations, while the extracted triflate was acid-free. All further radioiodinations were carried out with the extracted triflate. "Cold" iodination of the non-extracted triflate appeared to be more successful than radioiodination, as HPLC analysis of the reaction mixture indicated complete conversion of the triflate to the iodinated compound.

The radioiodination reactions using the triflate **2.85** were generally carried out at room temperature, using 1 - 5 mg triflate in 0.2 ml acetone. The reaction product was fairly pure, and its HPLC retention time was similar to that obtained from the tosylate. Despite the high HPLC

yields obtained, the recovered radiochemical yields, after purification, were somewhat inconsistent. This was because of the high amount of residual activity that often remained in the reaction vials after the transfer of the reaction mixtures to the silica gel column. Radiochemical yields obtained under various reaction conditions are listed in Table 3.2.

TABLE 3.2 Radiochemical yields of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitro-imidazole **2.87** using 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85** as precursor

Mass triflate (mg)	Age triflate (days)	Reaction temperature (°C)	Reaction time (min.)	Radiochemical yield (HPLC) (%)	Recovered radiochemical yield (%)
1.5	1	room	75	69	69
1.5	8	50°C	15	93	77
3.0	2	room	10	94	67
5.0	8	room	10	83	40
1.0	15	room	25	95	50
2.0	22	room	20	86	80

It appears that short-term ageing of the triflate **2.85**, as well as its concentration in the reaction mixture, did not significantly influence the radiochemical yields. HPLC yields were fairly consistent, but recovered yields varied to some extent. The radiochemical purity of the final product was always higher than 98%, but, as with the tosylates, the product contained a trace of the triflate. The main feature of these results is that the triflate is a better precursor than the tosylate for the radiosynthesis of compounds of the type of [^{123}I]**2.87**. Milder reaction conditions can be used, and radiochemical yields are generally significantly higher. The only disadvantage of the triflate route is the limited shelf life of the solid triflate which has to be stored as a solution in an organic solvent such as dichloromethane. The tosylates, on the other

hand, are very stable in solid form over several months when stored at -10°C. It was for this reason that, in this study, tosylates were mostly preferred as precursors for radioiodinations.

3.4.5.3 Comparison of radioiodinations with 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxy-prop-2-yloxy)methyl]imidazole **2.83** and 2-methyl-4-nitro-1-[(1-trifluoromethane-sulphonyloxyprop-2-yloxy)methyl]imidazole **2.85**

To illustrate the much higher reactivity of triflates, the tosylated precursor **2.83** and its triflate analogue **2.85** were radioiodinated under identical reaction conditions. The precursor concentration in acetone was 1.5 mg / 0.2 ml. Both reactions were initially carried out at 50°C. The temperature of the tosylate reaction mixture was increased during the course of the radioiodination. The radiochemical yields as determined by HPLC are shown in Table 3.3.

TABLE 3.3 Comparison of the radiochemical yield of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87** from tosylate **2.83** and triflate **2.85** as precursors, as determined by HPLC

Reaction temperature (°C)	Reaction time (min.)	Radiochemical yield of [^{123}I]**2.87** (%)	
		From tosylate **2.83**	From triflate **2.85**
50	15	0.5	93
50	30	0.5	93
100	15	2.3	-
100	30	3.3	-
140	15	35	-
140	35	40	-
160	10	41	-

It is evident that the rate of radioiodination is strongly dependent on the temperature when the tosylate is used as the precursor. In contrast, the radioiodination using the triflate proceeds at a

high rate at a relatively low temperature. For commercial purposes, where high yields and short reaction times are required, the triflate would be the preferred precursor.

3.4.6 Radiosynthesis of 1-[(2-[^{123}I]iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92**

According to Fleet (1989), nucleophilic substitution at a secondary carbon in carbohydrates requires the use of a triflate, rather than a mesylate or a tosylate as leaving group since the oxygen atom in the carbohydrate ring, situated in a β-position with respect to the leaving group, dramatically slows down S_N reactions. The radiosynthesis of [^{123}I]**2.92** also requires a nucleophilic substitution of a leaving group at a secondary carbon, which is in a *β*-position relative to an oxygen atom. It was therefore decided to prepare this compound by using only the triflate precursor 2-methyl-4-nitro-1-[(2-trifluoromethanesulphonyloxyprop-1-yloxy)methyl]imidazole **2.90**. All the labelling reactions were carried out at room temperature, with the precursor concentration varying between 1 and 9 mg / 0.2 ml acetone. Radiochemical yields (RCYs) and purities are listed in Table 3.4.

TABLE 3.4 Radiochemical yields and purities of 1-[(2-[^{123}I]iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92** from the triflate precursor **2.90** as starting material

Mass triflate (mg)	Age triflate (days)	Reaction time (min)	HPLC yield (%)	Recovered RCY (%)	Radiochemical purity of the final product (%)
3.0	1	50	46	30	91
9.0	1	45	45	29	91
5.0	8	53	43	20	84
1.0	15	40	42	20	84
1.0	22	20	26	10	77

The results in Table 3.4 reflect the much lower radiochemical yield of [^{123}I]**2.92** obtained from the triflate precursor **2.90**, in comparison to the yield of [^{123}I]**2.87** obtained from the triflate

precursor **2.85** shown in Table 3.2. This was mainly because of the formation of an unknown volatile radiochemical impurity during the labelling reaction between **2.90** and radioiodide. The removal of this impurity, by concentrating the reaction mixtures to dryness before the product was purified by column chromatography over silica gel, resulted in activity losses ranging from 14% to 41%. By omitting the concentration step, it was possible to isolate the impurity in a radiochemical yield of 37 to 52%. Radio-HPLC analysis of such an isolated fraction showed the presence of three components [(Method 2 (b)]. The first component had the same retention time as the radioiodide, but it is unlikely that it could have been the iodide because iodide is not volatile. It is possible that it could have been molecular iodine, which is volatile. This, however, would imply that the original radioiodide had been partially oxidised during the labelling reaction, to give volatile radioiodine that did not take part in a nucleophilic substitution reaction. On the other hand, the complete absence of oxidising agents in the reaction mixture, as well as the apparent absence of conditions that could promote any form of oxidation, makes this assumption questionable. The second component appeared approximately halfway between the first component and the product [^{123}I]**2.92** in the chromatogram. The third component was a trace of the target compound [^{123}I]**2.92**. The relative proportions of the three components were approximately 51:40:7, respectively. Evaporation to dryness of this fraction resulted in an approximate 85% loss of its radioactivity. Radio-HPLC analysis of the dried residue indicated the partial disappearance of the first two components, with a concurrent enrichment in [^{123}I]**2.92**. This confirmed that the product was not volatile. No further attempt was made to identify the first and the second component of the isolated fraction.

The radiochemical yields of 1-[(2-[^{123}I]iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92** did not appear to be related to the concentration of the precursor. However, despite the shorter reaction time employed in the reaction listed as the last entry in Table 3.4, there seemed to be a general trend of decreasing yield, as well as decreasing radiochemical purity of [^{123}I]**2.92**, with ageing of the triflate precursor **2.90**. This is in agreement with the observed decomposition of the precursor **2.90** over time (see paragraph 2.3.3.6), and is in contrast to the consistent yields of [^{123}I]**2.87** obtained despite ageing of the triflate **2.85**, as shown in Table

3.2. These results illustrate the disadvantage of using a triflate as precursor in which the triflate group is bound to a secondary carbon atom.

The average isomeric purity of the purified product [^{123}I]**2.92** was not as high as that of its structural isomer [^{123}I]**2.87**. This was mainly because of the presence of a trace of [^{123}I]**2.87** that was always present in [^{123}I]**2.92**. A possible reason is that the triflate **2.90**, the precursor for [^{123}I]**2.92**, contained a trace of its isomer, the triflate **2.85**, the precursor for [^{123}I]**2.87**. The authenticity of these two radioiodinated isomers was confirmed by the corresponding retention times of their respective cold iodinated analogues in an HPLC chromatogram.

3.4.7 Radiosynthesis of 1-[(1-benzoyloxy-3-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.99** and its conversion to 1-[(1-hydroxy-3-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100**

3.4.7.1 Attempted radiosynthesis of [^{123}I]**2.99** from the triflate 1-[(1-benzoyloxy-3-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.95** as precursor

Because of the unsuccessful isolation of the pure triflate **2.95** in the synthesis of this precursor (see Section 2.3.3.7), attempts were made to carry out the radioiodinations using the crude reaction mixtures after their dissolution in acetone. Radiochemical yields as determined by HPLC, however, were very low (less than 10%). This is reminiscent of the modest yields of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87** obtained from the insufficiently purified 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85** (see paragraph 3.4.5.2). Attempts to synthesise [^{123}I]**2.99** from the triflate precursor were therefore abandoned.

3.4.7.2 Radiosynthesis of [^{123}I]**2.99** from the tosylate 1-[(1-benzoyloxy-3-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.97** as precursor

The radiosynthesis of [^{123}I]**2.99** from the tosylate precursor **2.97** proved to be fairly successful. Reaction conditions were similar to those used for all the other radioiodide-for-tosylate exchange reactions. A reaction temperature of 150°C and a reaction time of 30 minutes resulted in a high HPLC yield (94%). The retention time of the product corresponded to that of the cold iodinated compound 1-[(1-benzoyloxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.99**. Alkaline hydrolysis of [^{123}I]**2.99** gave a compound having a retention time corresponding to that of the cold iodinated compound 1-[(1-hydroxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100**. The hydrolysis was effectively carried out with sodium hydroxide in aqueous ethanol at room temperature for 30 minutes. In most cases radio-HPLC analysis of the hydrolysis mixture indicated almost complete conversion of [^{123}I]**2.99** to [^{123}I]**2.100** after 10 minutes. This step also led to some de-iodination, as well as the formation of a slightly less polar radiochemical impurity (3%). Wada *et al.* (2000) made use of a cation-exchange resin to neutralise the hydrolysis mixture before evaporating it to dryness. In the present investigation, this neutralisation step was initially omitted, resulting in substantial de-iodination of [^{123}I]**2.100** (increase of the free radioiodide content from 8% to 78%). This did not happen when the hydrolysis mixture was pre-treated with a cation exchange resin. The neutralisation step was followed by the usual purification over silica gel, using ethyl acetate as mobile phase. Recovered yields ranged from 48% to 62% of the initial activity input. The radiochemical purity of the purified product was approximately 96%, with the previously mentioned less polar compound as the major radiochemical impurity.

CHAPTER 4

EVALUATION OF THE RADIOCHEMICAL STABILITY OF THE LABELLED COMPOUNDS

As mentioned in the statement of objectives, only *in vitro* stability tests were carried out on the various labelled compounds with the aim to observe stability trends. The release of free radioiodide from the labelled compound over time was the criterion by which its stability was assessed. It was not planned to monitor the stability over short, regular time intervals, but rather to determine it over a short-term (3 to 5 hours) and a long-term (21 to 24 hours) period. Human blood serum was used as the test medium. The serum had been separated from the blood cells by means of centrifugation of a freshly drawn blood sample in a tube containing an anti-coagulating agent, and stored in sterile vials at 4°C. The labelled compound, after its chromatographic purification over silica gel, was evaporated to dryness with a stream of nitrogen, and the dried residue dissolved in a small amount of the serum by means of a strong vortex action. Additional fresh serum was added and a small aliquot of the solution was taken to represent the sample at time zero. The rest of the serum solution was then incubated in an incubator oven or water bath set at 37°C. Aliquots were taken at the indicated time intervals.

4.1 EVALUATION OF THE RADIOCHEMICAL STABILITY OF THE RADIOIODINATED COMPOUNDS PREPARED IN THE PILOT STUDY

The stability results of the radioiodinated analogues of the iodinated compounds **2.4**, **2.7**, **2.12**, **2.15**, **2.19**, **2.22**, **2.26** and **2.30** are presented in Figure 4.1. To verify the accuracy of some of the data, TLC and HPLC were both utilised in a few experiments to determine the amount of released radioiodide. No other breakdown products were observed. The results in Fig. 4.1 represent the average of the data, obtained from both TLC and HPLC, from two or more experiments. No significant differences were observed between the data obtained from these

two methods. Except for compounds **2.26** and **2.30**, which were regarded as approximately crude references, the combined data obtained by the two methods supplied sufficiently reproducible data per compound. The results obtained at three hours reflect the short-term radiochemical stability, while those obtained at 21 hours reflect the long-term stability of the radioiodinated compounds.

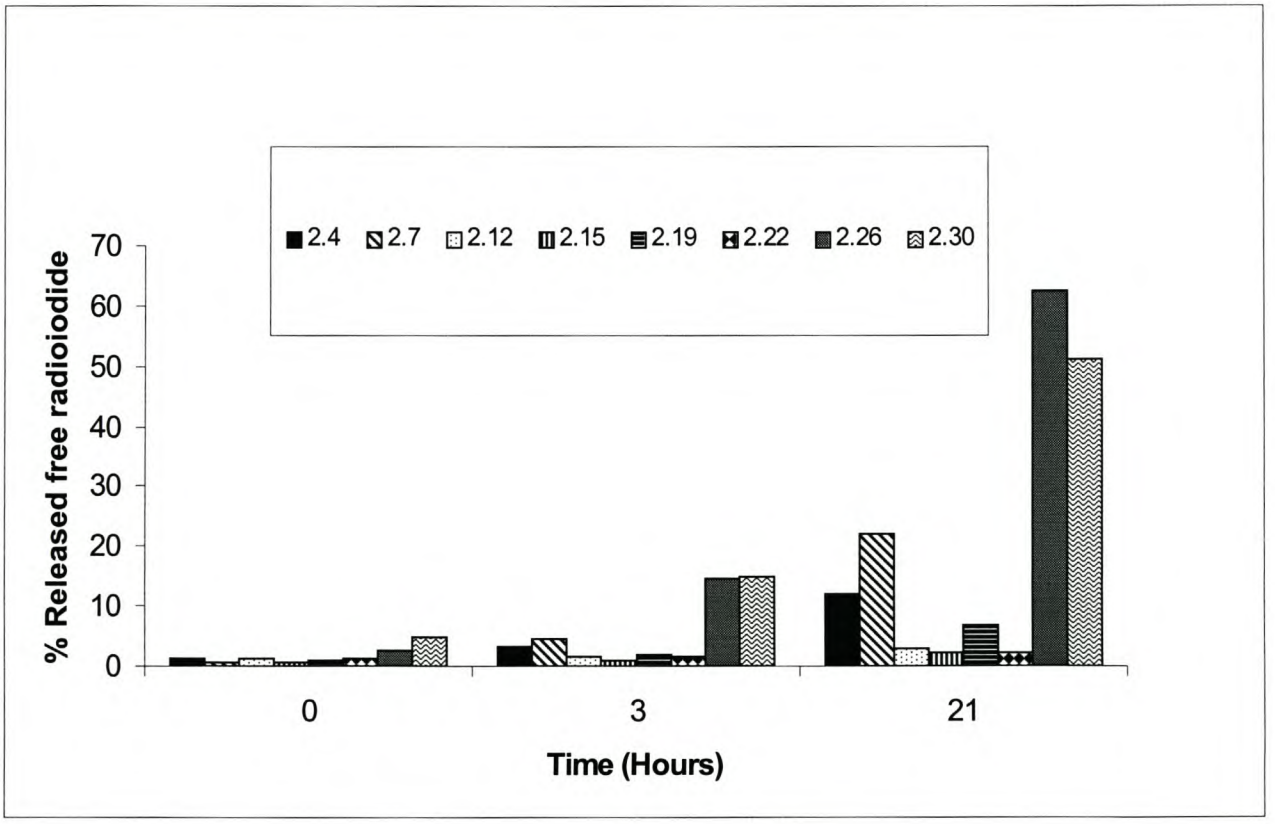


Figure 4.1. Release of radioiodide from the ^{131}I-labelled β-iodoalkylether derivatives 1-iodo-2-phenoxyethane **2.4**, 1-benzyloxy-2-iodoethane **2.7**, 1-iodo-2-phenoxypropane **2.12**, 2-iodo-1-phenoxypropane **2.15**, 1-iodo-2-phenoxy-butane **2.19**, 1-iodo-2-methoxy-2-phenylethane **2.22**, 1-iodo-3-phenoxypropane **2.26** and 1-iodo-2-phenylethane **2.30** versus incubation time in human blood serum at 37 °C.

The significantly higher degree of de-iodination exhibited by the compounds lacking a β-oxygen atom, 1-iodo-3-phenoxypropane **2.26** and 1-iodo-2-phenylethane **2.30**, in comparison with the β-iodoethers 1-iodo-2-phenoxyethane **2.4**, 1-benzyloxy-2-iodoethane **2.7**, 1-iodo-2-

phenoxypropane **2.12**, 2-iodo-1-phenoxy-propane **2.15**, 1-iodo-2-phenoxy-butane **2.19** and 1-iodo-2-methoxy-2-phenylethane **2.22**, confirms the stabilising effect of a β-oxygen atom in an iodoalkoxy unit. It is clear that compounds **2.4**, **2.7**, **2.12**, **2.15**, **2.19** and **2.22** are all reasonably stable during the first three hours of incubation, while those containing a branched structure in the aliphatic chain (**2.12**, **2.15**, **2.19** and **2.22**) show even slightly superior stability. After 21 hours the straight chain compounds **2.4** and **2.7** show higher degrees of de-iodination than compounds **2.12**, **2.15**, **2.19** and **2.22**. This increased stability of compounds **2.12**, **2.15**, **2.19** and **2.22** is most probably caused by the steric effect of the branched structure of the aliphatic chain. The position of the branched structure (alpha or beta with respect to the ether oxygen as displayed in compounds **2.12** and **2.15**, respectively) does not appear to exert a significant influence on the stabilising effect. The influence of the size of the group is somewhat ambiguous. 1-Iodo-2-phenoxy-butane **2.19**, having an α-ethyl group, displays a slightly higher degree of de-iodination after 21 hours than 1-iodo-2-phenoxypropane **2.12**, having an α-methyl group, suggesting that the more space-filling group causes more instability. On the basis of this argument, it would be expected that 1-iodo-2-methoxy-2-phenylethane **2.22**, having a bigger α-phenyl group, would be less stable than **2.12**, which contains a smaller α-methyl group. However, **2.22** displays a similar degree of stability to that of **2.12**. A possible explanation for this ambiguity is given in the following paragraph.

Another feature of the stability results is the significantly higher degree of de-iodination after 21 hours displayed by the benzylic ether 1-benzyloxy-2-iodoethane **2.7** as opposed to that of the phenolic ether 1-iodo-2-phenoxyethane **2.4**. This can possibly be explained in terms of the mechanism by which dehalogenation of haloalkanes occur. According to Anders and Pohl (1985), the *in vivo* metabolism of these compounds generally involves a preferred hydroxylation at the halogen-bearing carbon atom, followed by an elimination of the halide ion. Electron-donating groups in such a molecule would stabilise a carbenium ion intermediate in the metabolic hydroxylation process by means of a resonance effect, while electron removal by an inductive mechanism destabilises such intermediates (*French et al., 1991*). The β-heteroatom is too distant to have a positive resonance effect, but can provide a deactivating

inductive effect, thereby resulting in diminished de-halogenation (*French et al., 1991*). When an electron-withdrawing group such as a phenyl group is present in the vicinity of the halogen-bearing carbon, it can assist the β-heteroatom in exerting a -I effect, resulting in increased stability of the carbon-halogen bond. This effect should be diminished with an increase in the distance between the phenyl group and the halogen-bearing carbon. Assuming that the *in vivo* dehalogenation process as proposed by Anders and Pohl is also, to some extent, applicable to *ex vivo* processes, the following arguments can be presented. In the benzylic ether **2.7** the phenyl group is one atom further removed from the halogen-bearing carbon, as opposed to the one in the phenolic ether **2.4**. The carbenium intermediate in **2.7** will therefore experience a diminished net inductive effect and therefore less destabilisation compared to **2.4**. Consequently, compound **2.7** will be more prone to dehalogenation in comparison to **2.4**. These arguments might also explain the higher than expected degree of stability shown by 1-iodo-2-methoxy-2-phenylethane **2.22**, in which the phenyl group is attached to the carbon adjacent to the halogen-bearing carbon, resulting in a higher net inductive effect, more destabilisation of the carbenium ion intermediate, and therefore less de-iodination. It is assumed that this effect outweighs the negative influence of the space-filling property of the phenyl group on the radiochemical stability of **2.22**.

4.2 EVALUATION OF THE RADIOCHEMICAL STABILITY OF THE RADIOIODINATED BENZAMIDE DERIVATIVES

The results of the evaluation of the stability of the radioiodinated analogues of compounds **2.42**, **2.44**, **2.46** and **2.48** are presented in Figure 4.2. The release of free radioiodide was determined by means of HPLC analysis only. No other breakdown products were observed. All the data supplied represent the averages from the results of two to four experiments per compound.

The stability trends of the compounds displayed in Figure 4.2 are in agreement with those given in Figure 4.1. The straight-chain *β*-iodoethoxy moiety in 2-(2-iodoethoxy)-3-methoxy-*N*-

(2-piperidin-1-ylethyl)benzamide **2.42** de-iodinates at a faster rate than the branched chain moieties in 2-(1-iodoprop-2-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.44** and 2-(2-iodoprop-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.46**, confirming the stabilising effect of a branched structure in such a unit. Apparently there is no significant difference between the stabilities of the isomers **2.44** and **2.46**, in agreement with the stability trends displayed by 1-iodo-2-phenoxypropane **2.12** and 2-iodo-1-phenoxypropane **2.15** in Figure 4.1. Another significant feature is that, over the long term (21 to 24 hours), the extent of de-iodination (~50%) of the straight-chain moiety in the benzamide **2.42** (Figure 4.2) is significantly higher than that of the same moiety in the model compound 1-iodo-2-phenoxyethane **2.4** (Figure 4.1) (~14%). The reason for this phenomenon is not clear, but the higher instability of **2.42** could perhaps be related to the presence of the nearby amide and/or methoxy groups on the aromatic ring. If this assumption is correct, it illustrates the influence of the chemical environment on the stability of these moieties. The superior long-term stability displayed by the iodovinyl moiety in 2-(3-iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.48** is to be expected. However, the medium-term stability of **2.44** and **2.46** compares well with that of **2.48**.

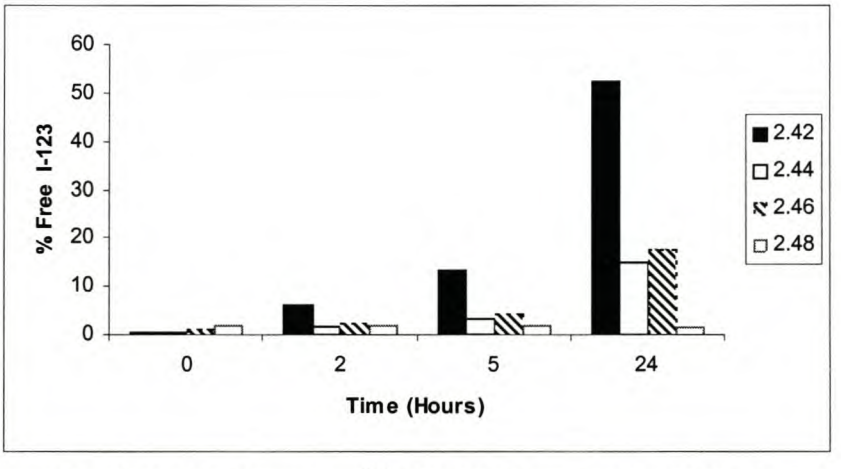
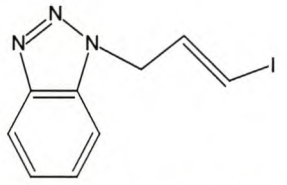


Figure 4.2. Release of radioiodide from the ^{123}I-labelled benzamide derivatives 2-(2-iodoethoxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.42**, 2-(1-iodoprop-2-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.44**, 2-(2-iodoprop-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.46** and 2-(3-iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.48** versus incubation time in human blood serum at 37 °C.

4.3 EVALUATION OF THE RADIOCHEMICAL STABILITY OF THE RADIOIODINATED HETEROCYCLIC AMINE DERIVATIVES

The stability evaluation results of the radioiodinated analogues of compounds **2.66b**, **2.70**, **2.74**, **2.78**, the reference compound 1-(3-iodo-2-propen-1-yl)benzotriazole, **2.87**, **2.92** and **2.100** are presented in Figures 4.3 to 4.5. The release of free radioiodide was determined only by means of HPLC analysis. No other breakdown products were observed. All data were obtained as averages from the results of two to five experiments per compound. The data for the ^{123}I-labelled 1-(3-iodo-2-propen-1-yl)benzotriazole, displayed in Figure 4.3, were obtained from another source (*Visser, 2002*).



1-(3-iodo-2-propen-1-yl)benzotriazole

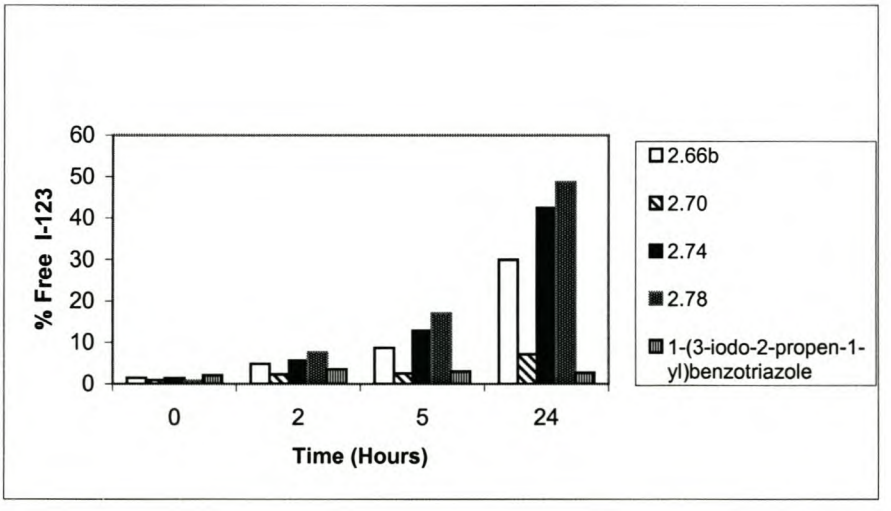


Figure 4.3 Release of radioiodide from the ^{123}I-labelled benzotriazole derivatives 1-[(2-iodoethoxy)methyl]benzotriazole **2.66b**, 1-[(1-iodoprop-2-yloxy)methyl]benzotriazole **2.70**, 1-(2-iodoprop-1-yl)benzotriazole **2.74**, 1-(3-iodo-2-methylprop-1-yl)benzotriazole **2.78** and 1-(3-iodo-2-propen-1-yl)benzotriazole versus incubation time in human blood serum at 37 °C.

The radiochemical stability trends of the β-iodoalkylether compounds displayed in Figure 4.3 are, once again, in agreement with those in Figures 4.1 and 4.2. The straight-chain *β*-iodoethoxy moiety in [^{123}I]**2.66b** de-iodinates at a faster rate than the branched chain moiety in [^{123}I]**2.70**, especially over the long term. A comparison of the rates of de-iodination between the straight chain moieties [^{123}I]**2.66b** (Fig. 4.3) and 2-(2-iodoethoxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.42** (Fig. 4.2) shows a lesser degree of de-iodination of the former than the latter. This difference in long-term stability is not as significant as that between **2.42** and 1-iodo-2-phenoxyethane **2.4** (Fig. 4.1), but could probably also be ascribed to the influence of the chemical environment. The significantly higher de-iodination rates exhibited by the *β*-oxygen-lacking moieties in [^{123}I]**2.74** and [^{123}I]**2.78**, in comparison with that shown by [^{123}I]**2.70**, are also in agreement with the stability trend shown by 1-[^{131}I]iodo-2-phenylethane **2.30** (Fig. 4.1). Apparently the branched structure on its own does not contribute significantly to the increased stability shown by a moiety of the type shown in **2.70**, but rather the combination of the branched structure and a *β*-oxygen. Compound **2.74**, in which the iodine atom is attached to a secondary carbon atom, also appears to de-iodinate at a slightly slower rate than **2.78**, in which the iodine atom is attached to a primary carbon atom. This difference, however, is not really significant, implying that the position of the iodine atom with respect to the branched methyl group does not have a significant influence on stability. This is also in agreement with the small differences in stability observed between the *β*-oxygen-containing 2-(1-iodoprop-2-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.44** and 2-(2-iodoprop-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.46**, as well as between 1-iodo-2-phenoxypropane **2.12** and 2-iodo-1-phenoxy-propane **2.15**. The apparent higher degree of de-iodination shown by the imidazole derivative 1-[(2-[^{123}I]iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92** in comparison to that of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87** in Figure 4.4 can be ascribed to the higher amount of free radioiodide present in **2.92** at time zero. The graphs in Figure 4.5 show that the rates of de-iodination of these two compounds are quite similar.

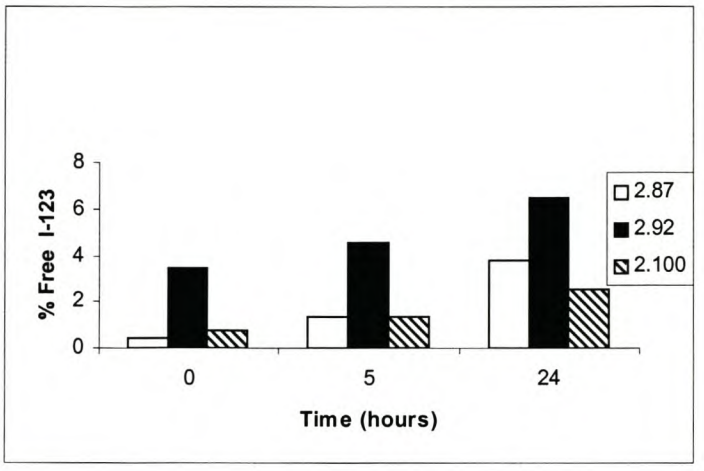


Figure 4.4 Release of radioiodide from the ^{123}I-labelled 2-methyl-4-nitroimidazole derivatives 1-[(1-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87**, 1-[(2-iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92** and 1-[(1-hydroxy-3-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100** versus incubation time in human blood serum at 37 °C.

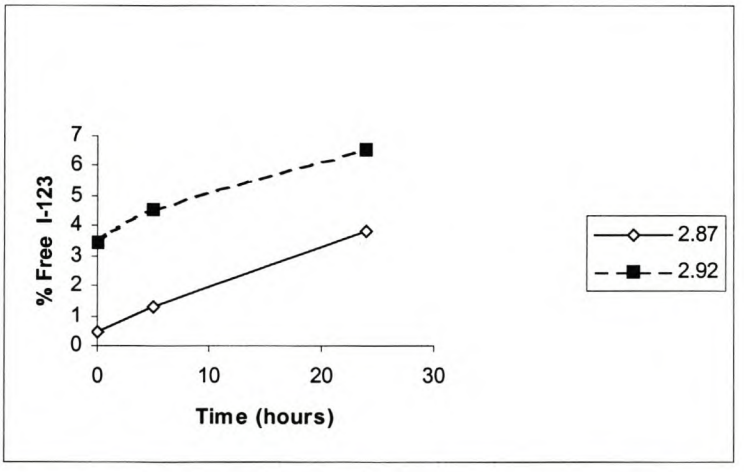


Figure 4.5 Comparative release of radioiodide from the ^{123}I-labelled 2-methyl-4-nitroimidazole derivatives 1-[(1-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87** and 1-[(2-iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92** versus incubation time in human blood serum at 37 °C.

The bar graphs in Figure 4.3 show that the stability of 1-[(1-iodoprop-2-yloxy)methyl]benzotriazole **2.70** compares favourably with that of 1-(3-iodo-2-propen-1-yl)benzotriazole, even over the long term. This is in contrast to the poorer long-term stability shown by the identical β-iodoethoxy moiety in the benzamide **2.44**, in comparison with that of

the iodovinyl moiety in the benzamide 2-(3-iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.48** (see Fig. 4.2). This difference in stability could probably again be ascribed to the influence of the chemical environment.

Finally, the de-iodination rates of the nitroimidazole derivatives 1-[(1-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87** and 1-[(1-hydroxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100** shown in Figure 4.4 are quite similar, suggesting that substituting a methyl group with a hydroxymethyl group as the side chain in a β-iodoethoxy moiety does not have a significant influence on the stability of such a moiety. It further suggests that a radioiodinated analogue of the radiofluorinated 1-[(1-fluoro-3-hydroxyprop-2-yloxy)methyl]-2-nitroimidazole **1.32** could also be developed as a radiopharmaceutical. Furthermore, the radioiodinated analogues of the nitroimidazoles **2.87** and **2.100** could be considered as possible infection- or inflammation-seeking agents, as their chemical structures bear resemblance to that of the established infection- or inflammation- seeking agent ornidazole **1.13** (see paragraph 1.2.2). They could also be utilised for imaging hypoxic tissues (*Wada et al., 2000*).

CHAPTER 5

SUMMARY AND FINAL CONCLUSIONS

The radiochemical stability of radiopharmaceuticals is a very important consideration in the radiopharmaceutical field. The retention of the incorporated radionuclide by its organic carrier is vital with regard to the correct diagnosis of diseases, as well as the elimination of unnecessary irradiation of healthy organs. This is especially applicable to radioiodinated radiopharmaceuticals, as the carbon-iodine bond is the weakest in the carbon-halogen series. Traditionally, radioiodine is incorporated into organic structures such as phenyl rings and vinyl prosthetic units that give a certain degree of stability to the bond. However, for reasons already mentioned, the need also exists for alternative structural units for the incorporation of radioiodine. The main objective of this study was to investigate the trends in radiochemical stability of various radioiodinated organic compounds, with particular emphasis on those compounds containing so-called iodinated prosthetic groups. The β-iodoethoxyl moiety was selected as the prosthetic group for investigation because it is a relatively novel prosthetic group in radiopharmaceutical chemistry.

The study was started by conducting a systematic investigation into the *in vitro* radiochemical stability of model compounds containing radioiodinated β-iodoethoxyl units and derivatives thereof, as well as the stability of reference compounds lacking a β-oxygen atom. Tosylated precursors suitable for labelling were synthesised, and their radioiodinated analogues were obtained in modest to fair radiochemical yields. Throughout this study, no deliberate attempts were made to optimise either chemical or radiochemical yields, as this was considered to be beyond the scope of this work. Stability evaluation was carried out *ex vivo*, under semi-physiological conditions. The results confirmed that aliphatic radioiodinated units are much less prone to de-iodination when they contain an oxygen atom in a β-position relative to the iodine atom. This investigation also showed for the first time that the introduction of a side

chain, in the form of a small unit such as a methyl group, into such a moiety further improves its stability, especially the long-term stability.

The study was extended to the investigation of the stability behaviour of a few selected prosthetic units incorporated into carrier molecules, such as phenolic benzamides and heterocyclic amines. Some of these carrier molecules were structurally similar to established radiopharmaceutical compounds. The synthesis of the tosylated precursors of the radioiodinated benzamides and heterocyclic amines were slightly more complex than the synthesis of the model compounds. The radiochemical evaluation of these radioiodinated compounds showed similar de-iodination trends to those shown by the radioiodinated model compounds. Furthermore, there were indications that the chemical environment of the β-iodoethoxyl units might influence their stability. The radiochemical stability of the stabilised β-iodoethoxyl units, especially those present in the heterocyclic amines, compares favourably with that of the "gold standard" iodovinyl units.

This work has shown that there is a promising future in utilising the β-iodoethoxyl unit in radiopharmaceutical chemistry, provided that the unit is stabilised as shown in this investigation. The evaluation of the radiochemical stability of these units *in vivo* is considered to be the following important step in the development of such compounds, because very little is known about their stability against metabolic processes in the body. Other aspects such as more practical radiolabelling methods should also be investigated.

CHAPTER 6

EXPERIMENTAL

6.1 GENERAL

Unless stated otherwise, all reagents and solvents were of analytical grade and were purchased from Aldrich Chemicals. These solvents and chemicals were used without distillation or further purification. Dichloromethane, acetonitrile and acetone were dried over 4Å molecular sieves. [^{131}I]iodine was obtained from Amersham Buchler (Germany). [^{123}I]iodine was produced at iThemba LABS, Faure, South Africa by the $^{127}I(p,5n)^{123}Xe \rightarrow {}^{123}I$ route and recovered as a no-carrier-added product in 0.01 M NaOH solution. The solutions containing [^{123}I]iodine were concentrated approximately 3 to 4 fold in order to increase the radioactivity concentration, which ranged between 240 mCi (8.88 GBq) and 400 mCi (14.8 GBq) per milliliter at the time of labelling. For the pilot study work, HPLC, using isocratic elution, was carried out using a Knauer or Waters Model 510 pump. Reversed phase columns (MultoSorb RP18, LichroCart RP18 and Synergi C_{12}) were used. For the work on the benzamides and heterocyclic amines, gradient elution was carried out using a Perkin Elmer Series 200 LC Binary Pump equipped with a Rheodyne Model 7125 injector, and a Phenomenex Luna 5µ C_{18} (250 × 4.6 mm) reversed-phase column. All gradient elutions were linear. HPLC pumps were connected to a UV detector (254nm) coupled in series with a radiation flow detector with a NaI(Tl) crystal and the necessary electronic devices (ORTEC). Data were recorded and processed on a Hewlett Packard Series 3394 integrator. GC-MS analysis of bromopropanol preparations was done on a Carlo Erba QMD-1000 instrument, using a GC column (40 m) of fused silica coated with PS-089 (5% phenyl). The purity analysis of bromopropanol preparations was carried out on a Hewlett Packard Series 6890 gas chromatograph. A DB 1701 capillary column (50 m × 0.32 mm) was used. The identities of the individual bromopropanol isomers were verified by comparison of their respective retention times with those of a commercial isomer mixture with

known composition. Analytical thin layer chromatography (TLC) was performed using silica gel UV_{254} pre-coated aluminium-supported plates. Developed plates were inspected under a Model UVGL-58 Mineralight®Lamp (UV-254/366 nm) at 254 nm. All silica gel column chromatography purification procedures were done by means of descending chromatography on packed columns (inside diameter = 10 mm). The silica gel (particle size 0.063 – 0.2 mm) was purchased from Fluka. Collected fractions were developed on TLC alongside an aliquot of the crude reaction mixture. The most intense spot in the TLC chromatogram of the reaction mixture was regarded as the main product. Reaction mixtures and fractions obtained from preparative column chromatography were concentrated or evaporated to dryness under reduced pressure on a rotary evaporator (Büchi). Radioactivity measurements were done in a radionuclide assay calibrator (Vinten). Autoradiography (inspection of radio-TLC plates) was performed on an InstantImager System (Packard). Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. For the compounds synthesized in the pilot study, proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker 200 MHz instrument. No ^{13}C NMR data were recorded for these compounds. ^{1}H and ^{13}C NMR spectra of the benzamides and heterocyclic amines were recorded on either a 300MHz Varian VXR spectrometer equipped with a Varian magnet (7.0 T) or a 600MHz Varian Unity Inova spectrometer equipped with an Oxford magnet (14.09 T). Standard pulse sequences were used to obtain ^{1}H and ^{13}C NMR spectra. Unless stated otherwise, all spectra were recorded using $CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal reference. ^{13}C NMR spectra were processed by using the chemical shift value of the centre of the three peak multiplet of chloroform-d (77.0 ppm) as internal reference. Mass spectra were recorded on an AMD 604 double focussing mass spectrometer. Mass spectra were not recorded for compounds prepared in the pilot study. Stability experiments were carried out in a water-jacketed incubator oven (Forma Scientific, Ohio, U.S.A.).

6.2 CHROMATOGRAPHIC METHODS

6.2.1 HPLC: Binary gradient elution

In all of these methods, a Luna 5μ C_{18} (250 × 4.6 mm) reversed phase column was used. All gradient elutions were linear.

Method 1

A = 0.1% Aqueous triethylamine, B = Acetonitrile

STEP	TIME	FLOW RATE	MOBILE PHASE COMPOSITION
1.	5 min.	1ml/min.	40% A 60% B
2.	10 min.	1ml/min.	20% A 80% B
3.	5 min.	1ml/min.	0% A 100% B
4.	5 min.	1ml/min.	0% A 100% B

Method 2

(a) A = 0.1% Aqueous triethylamine, B = Acetonitrile

(b) A = 0.1% Aqueous trifluoroacetic acid, B = Acetonitrile

STEP	TIME	FLOW RATE	MOBILE PHASE COMPOSITION
1.	3 min.	1ml/min.	70% A 30% B
2.	5 min.	1ml/min.	50% A 50% B
3.	5 min.	1ml/min.	40% A 60% B
4.	5 min.	1ml/min.	0% A 100% B
5.	15 min.	1ml/min.	0% A 100% B

Method 3

(a) A = 0.1% Aqueous trifluoroacetic acid, B = Acetonitrile

(b) A = 0.01M Phosphate buffer (pH 2), B = Acetonitrile

STEP	TIME	FLOW RATE	MOBILE PHASE COMPOSITION	
1.	15 min.	1ml/min.	70% A	30% B
2.	5 min.	1ml/min.	0% A	100 % B

Method 4

A = 0.1% Aqueous trifluoroacetic acid, B = Acetonitrile

STEP	TIME	FLOW RATE	MOBILE PHASE COMPOSITION	
1.	15 min.	1ml/min.	60% A	40% B
2.	5 min.	1ml/min.	0% A	100 % B
3.	5 min.	1ml/min.	0% A	100 % B

Method 5

A = 0.1% Aqueous trifluoroacetic acid, B = Acetonitrile

STEP	TIME	FLOW RATE	MOBILE PHASE COMPOSITION	
1.	15 min.	1ml/min.	50% A	50% B
2.	5 min.	1ml/min.	0% A	100 % B
3.	5 min.	1ml/min.	0% A	100 % B

Method 6

A = 0.1% Aqueous trifluoroacetic acid, B = Acetonitrile

STEP	TIME	FLOW RATE	MOBILE PHASE COMPOSITION	
1.	5 min.	1ml/min.	90% A	10% B
2.	5 min.	1ml/min.	70% A	30 % B
3.	5 min.	1ml/min.	50% A	50 % B
4.	5 min.	1ml/min.	0% A	100% B

Method 7

A = 0.1% Aqueous trifluoroacetic acid, B = Acetonitrile

STEP	TIME	FLOW RATE	MOBILE PHASE COMPOSITION	
1.	15 min.	1ml/min.	80% A	20% B
2.	3 min.	1ml/min.	0% A	100 % B
3.	5 min.	1ml/min.	0% A	100 % B

6.2.2 HPLC: Isocratic elution

Method 8

Stationary phase: Multosorb C_{18} (300 × 4.6 mm) reversed phase

Mobile phase: Acetonitrile/water = 55:45

Flow rate: 1 ml/min

Method 9

Stationary phase: C_{12} (150 × 4.6 mm) reversed phase

Mobile phase: Acetonitrile/water = 63:37

Flow rate: 1 ml/min

6.2.3 TLC: Method number and mobile phase

Method 1: Hexane:chloroform = 70:30 (v/v)

Method 2: Hexane:acetone = 90:10 (v/v)

Method 3: Hexane:acetone = 80:20 (v/v)

Method 4: Diisopropylether:methanol:25% aqueous ammonia = 80:20:1 (v/v/v)

Method 5: Diisopropylether:isopropanol:25% aqueous ammonia = 85:15:0.5 (v/v/v)

Method 6: Petroleum ether:ethyl acetate = 1:1 (v/v)

Method 7: Petroleum ether:ethyl acetate = 2:1 (v/v)

Method 8: Petroleum ether:ethyl acetate = 3:1 (v/v)

Method 9: Ethyl acetate

Method 10: Ethyl acetate:petroleum ether = 3:1 (v/v)

Method 11: Ethyl acetate:methanol = 20:1 (v/v)

Method 12: Hexane:ethanol = 65:35

6.2.4 Gas Chromatography

Method 1

Starting temperature:	40 °C
Hold time:	0 min.
Oven Ramp:	4 °C / min.
Final temperature:	250 °C
Hold time:	15 min.
Inlet temperature :	200 °C
Carrier gas:	Helium
Detector type:	FID
Detector temperature:	275 °C

Retention times: 1-Bromo-2-propanol = 16.5 min.
2-Bromo-1-propanol = 17.8 min.

6.3 COLD SYNTHESES

6.3.1 Synthesis of β-Iodoalkylether Derivatives and Reference Compounds

In this section the yields of the iodinated compounds obtained in the last step of the synthesis were in most cases not recorded because of the small amounts of products that were recovered.

2-Phenoxyethyl p-*toluenesulphonate* (**2.3**)

A mixture of β-bromophenetole **2.1** (0.9 g, 4.5 mmol) and HMPT containing 15% water (25 ml) was heated in an oil bath at 130°C for 6 h, cooled to room temperature, diluted with water (70 ml) and extracted with diethylether (3 × 40 ml). The combined ether extracts were washed with water (2 × 25 ml), dried over anhydrous sodium sulphate, and the drying agent filtered off. Concentration of the filtrate under reduced pressure at 35°C gave the crude product, 2-phenoxyethanol **2.2** (0.44g), as an oil, which was not further purified. A portion of **2.2** (0.2 g) was mixed with a solution of *p*-toluenesulphonyl chloride (tosyl chloride) (0.31 g, 1.6 mmol) in dry pyridine (2 ml) and the mixture was refrigerated overnight at 4°C. The reaction mixture was subsequently poured into water (20 ml) and extracted with $CHCl_3$ (2 × 15 ml). The combined chloroform extract was washed with 0.1N H_2S0_4 (2 × 15 ml) and water (2 × 15 ml), dried over anhydrous sodium sulphate, and the drying agent filtered off. Concentration of the filtrate under reduced pressure at 95°-100°C gave 2-phenoxyethyl *p*-toluenesulphonate **2.3** (0.33 g, 55%). TLC (Method 1) showed a single spot. The product was therefore used without further purification. ^{1}H NMR (200 MHz) δ: 2.49 (s, 3H), 4.18 (t, 2H), 4.41 (t, 2H), 6.8-7.9 (m, 9H, aromatic protons).

1-Iodo-2-phenoxyethane (**2.4**)

Sodium iodide (41 mg, 0.27 mmol) was added to a solution of 2-phenoxyethyl *p*-toluenesulphonate **2.3** (70 mg, 0.24 mmol) in acetone (1 ml) and the mixture was refluxed and stirred on a hot plate for approximately 1 hour. The precipitate that had formed was filtered off and the filtrate evaporated to dryness under reduced pressure to give 1-iodo-2-phenoxyethane

2.4 (55 mg, 92%). ^{1}H NMR (200 MHz) δ: 3.47 (t, J = 6.5 Hz, 2H), 4.3 (t, J = 6.9 Hz, 2H), 6.93-7.38 (m, 5H, aromatic protons).

2-Benzyloxyethyl p-*toluenesulphonate* (**2.6**)

This compound was prepared from commercially available 2-benzyloxyethanol **2.5** (0.3 g, 2.0 mmol) according to the procedure described for the preparation of 2-phenoxyethyl *p*-toluenesulphonate **2.3**. Column chromatography over silica gel, using chloroform as mobile phase, gave pure 2-benzyloxyethyl *p*-toluenesulphonate **2.6** (0.27 g, 45%). ^{1}H NMR (200 MHz) δ: 2.47 (s, 3H), 3.72 (t, 2H), 4.23 (t, 2H), 4.52 (s, 2H), 7.3-7.9 (m, 9H, aromatic protons).

1-Benzyloxy-2-iodoethane (**2.7**)

This compound was prepared from 2-benzyloxyethyl *p*-toluenesulphonate **2.6** according to the procedure described for the preparation of 1-iodo-2-phenoxyethane **2.4**, except for heating the reaction mixture in an oil bath at 120°C. The mixture was chromatographed over silica gel with hexane/chloroform = 90/10 to give 1-benzyloxy-2-iodoethane **2.7**. ^{1}H NMR (200 MHz) δ: 3.33 (t, 2H), 3.76 (t, 2H), 4.63 (s, 2H), 7.3-7.4 (m, 5H, aromatic protons).

2-Phenoxy-1-propanol (**2.10**)

Potassium phenoxide **2.8** was prepared by heating a mixture of KOH (3 g, 53.5 mmol), phenol (5 g, 53.1 mmol) and ethanol (55 ml) at 70°C until the solid material was completely dissolved, followed by concentration to complete dryness under reduced pressure at 70°C. Potassium phenoxide **2.8** (1 g, 7.6 mmol) was stirred with acetonitrile (30 ml) in the presence of a small quantity of 18-Crown-6 (228 mg, 0.9 mmol) at 35°C. The resulting solution was stirred for 30 min. at room temperature. Ethyl 2-bromopropionate (2g, 11.1 mmol) was added to the solution of the phenoxide and the reaction mixture was stirred at 30°-35°C for approximately 2.5 hours, after which TLC (Method 2) indicated the complete consumption of the phenoxide. The potassium bromide that had been formed in the reaction was filtered off, and the filtrate was concentrated under reduced pressure at 60°C. Diethyl ether (40 ml) was added to the residue, the resulting precipitate was filtered off and the filtrate was extracted with water (15ml). The

supernatant ether layer, containing the organic product, was dried over anhydrous sodium sulphate, the drying agent was filtered off, and the filtrate was concentrated under reduced pressure at 100°C to complete dryness to give 1.5 g crude ethyl-2-phenoxypropionate **2.9**. A solution of **2.9** (1 g, 5.15 mmol) in diethylether (3 ml) was added dropwise to a stirred and chilled (5°C) suspension of lithiumaluminiumhydride (LAH) (0.4 g, 10.53 mmol) in freshly distilled tetrahydrofuran (THF) (10 ml). The reaction mixture was subsequently refluxed for 3h after which TLC (Method 2) showed some unreacted starting material. The addition of more LAH (0.4 g) and refluxing the reaction mixture for a further hour resulted in the complete conversion of **2.9** to the product. The excess LAH was decomposed by slow addition of 0.1N NaOH (10 ml) to the cooled and stirred reaction mixture, after which dichloromethane (50 ml) and water (70 ml) were added and the mixture vigorously stirred. The resulting emulsion was filtered through celite. The organic layer was separated, dried over anhydrous sodium sulphate, the drying agent was filtered off, and the filtrate was concentrated to dryness under reduced pressure at 60°C to give 2-phenoxy-1-propanol **2.10** (0.54 g, 69%). ^{1}H NMR (200 MHz) δ: 1.31 (d, J = 6.2 Hz, 3H), 2.37 (s, 1H), 3.75 (m, 2H), 4.54 (m, 1H), 7.0 (m, 3H, aromatic protons), 7.33 (m, 2H, aromatic protons).

2-Phenoxyprop-1-yl p-*toluenesulphonate* (**2.11**)

This compound was prepared from 2-phenoxy-1-propanol **2.10** (0.3 g, 2.0 mmol) according to the procedure described for the preparation of 2-phenoxyethyl *p*-toluenesulphonate **2.3** to give unpurified 2-phenoxyprop-1-yl *p*-toluenesulphonate **2.11** (0.58 g 96%). ^{1}H NMR (200 MHz) δ: 1.34 (d, J = 6.3 Hz, 3H), 2.48 (s, 3H), 4.16 (m, 2H), 4.59 (m, 1H), 6.83-7.82 (m, 9H, aromatic protons).

1-Iodo-2-phenoxypropane (**2.12**)

This compound was prepared from 2-phenoxyprop-1-yl *p*-toluenesulphonate **2.11** according to the procedure described for the preparation of 1-benzyloxy-2-iodoethane **2.7**, except for using dioxane/acetone (1:1) as solvent, to give 1-iodo-2-phenoxypropane **2.12**. ^{1}H NMR (200 MHz) δ: 1.52 (d, J = 6.1 Hz, 3H), 3.38 (m, 2H), 4.44 (m, 1H), 6.93-7.38 (m, 5H, aromatic protons).

1-Phenoxyprop-2-yl p-*toluenesulphonate* (**2.14**)

This compound was prepared from commercially available 1-phenoxypropan-2-ol **2.13** (0.5 g, 3.3 mmol) according to the procedure described for the preparation of 2-phenoxyethyl *p*-toluenesulphonate **2.3**. The crude product was crystallised from methanol to give pure crystalline 1-phenoxyprop-2-yl *p*-toluenesulphonate **2.14** (0.56 g, 56%), m.p. 100-101°C, ^{1}H NMR (200 MHz) δ: 1.47 (d, J = 6.5 Hz, 3H), 2.48 (s, 3H), 4.0 (m, 2H), 4.92 (m, 1H), 6.71-7.87 (m, 9H, aromatic protons).

2-Iodo-1-phenoxypropane (**2.15**)

This compound was prepared from 1-phenoxyprop-2-yl *p*-toluenesulphonate **2.14** according to the procedure described for the preparation of 1-iodo-2-phenoxyethane **2.4**. The reaction mixture was heated and stirred on a hot plate for 4 hours, and the product was chromatographed as described for the purification of 1-benzyloxy-2-iodoethane **2.7** to give 2-iodo-1-phenoxypropane **2.15**. ^{1}H NMR (200 MHz) δ: 2.04 (d, J = 6.9 Hz, 3H), 4.09 (m, 1H), 4.35 (m, 2H), 6.93-7.38 (m, 5H, aromatic protons).

2-Phenoxy-1-butanol (**2.17**)

A solution of 2-phenoxybutyric acid (1 g, 5.6 mmol) in diethylether (3 ml) was added dropwise to a stirred and cooled (ice bath) suspension of LAH (0.64 g, 16.9 mmol) in dry THF (16 ml). The reaction mixture was subsequently refluxed for 1.5 hours after which TLC (Method 3) showed complete consumption of the starting material. The reaction mixture was worked up as described for the preparation of 2-phenoxy-1-propanol **2.10**. The organic extract was dried over anhydrous sodium sulphate, the drying agent was filtered off, and the filtrate was concentrated to dryness under reduced pressure at 45°C to give 2-phenoxy-1-butanol **2.17** (0.88 g, 96%). ^{1}H NMR (200 MHz) δ: 1.0 (t, J = 7.5 Hz, 3H), 1.75 (m, 2H), 1.98 (m, 1H), 3.83 (m, 2H), 4.34 (m, 1H), 6.90-7.36 (m, 5H, aromatic protons).

2-Phenoxybut-1-yl p-*toluenesulphonate* (**2.18**)

This compound was prepared from 2-phenoxy-1-butanol **2.17** (0.2 g, 1.2 mmol) according to the procedure described for the preparation of 2-phenoxyethyl *p*-toluenesulphonate **2.3**. The crude product was purified by column chromatography over silica gel, using chloroform as mobile phase, to give pure 2-phenoxybut-1-yl *p*-toluenesulphonate **2.18** (0.23 g, 59%). ^{1}H NMR (200 MHz) δ: 0.98 (t, J = 7.4 Hz, 3H), 1.75 (m, 2H), 2.47 (s, 3H), 4.15 (m, 2H), 4.37 (m, 1H), 6.81-7.81 (m, 9H, aromatic protons).

1-Iodo-2-phenoxybutane (**2.19**)

This compound was prepared from 2-phenoxybut-1-yl *p*-toluenesulphonate **2.18** according to the procedure described for the preparation of 1-benzyloxy-2-iodoethane **2.7**, using acetone/dioxane (3:1) as solvent. The reaction mixture was heated for 3.5 hours in an oil bath at 120°C, and the product was purified by column chromatography over silica gel, using hexane/chloroform (70/30) as mobile phase, to give 1-iodo-2-phenoxybutane **2.19**. ^{1}H NMR (200 MHz) δ: 1.06 (t, J = 7.4 Hz, 3H), 1.91 (m, 2H), 3.39 (m, 2H), 4.17 (m, 1H), 6.94-7.38 (m, 5H, aromatic protons).

2-Methoxy-2-phenylethyl p-*toluenesulphonate* (**2.21**)

This compound was prepared from commercially available 2-methoxy-2-phenylethanol **2.20** (1.0 g, 6.6 mmol) according to the procedure described for the preparation of 2-phenoxyethyl *p*-toluenesulphonate **2.3**. The crude product was crystallised from hexane/ethanol (3:1) to give pure, crystalline 2-methoxy-2-phenylethyl *p*-toluenesulphonate **2.21** (1.7 g, 85%). No melting point data was obtained for this compound. ^{1}H NMR (200 MHz) δ: 2.50 (s, 3H), 3.27 (s, 3H), 4.12 (m, 2H), 4.45 (m, 1H), 7.25-7.44 (m, 7H, aromatic protons), 7.76-7.85 (m, 2H, aromatic protons).

1-Iodo-2-methoxy-2-phenylethane (**2.22**)

This compound was prepared from **2.21** according to the procedure described for the preparation of 1-iodo-2-phenoxyethane **2.4**, except for heating the reaction mixture in an oil

bath at 180°C. The crude product was purified by column chromatography over silica gel, using hexane/chloroform (90:10) as mobile phase, to give 1-iodo-2-methoxy-2-phenylethane **2.22**. ^{1}H NMR (200 MHz) δ: 3.33-3.42 (m, 5H), 4.34 (m, 1H), 7.32-7.46 (m, 5H, aromatic protons).

3-Phenoxyprop-1-yl p-*toluenesulphonate* (**2.25**)

The precursor for the tosylate **2.25**, 3-phenoxy-1-propanol **2.24**, was prepared from 1-bromo-3-phenoxypropane **2.23** (0.5 g, 2.3 mmol) according to the procedure described for the preparation of 2-phenoxyethanol **2.2** from β-bromophenetole **2.1**. The target tosylate **2.25** was prepared from crude **2.24** (0.3 g, 1.9 mmol) according to the procedure described for the preparation of 2-phenoxyethyl *p*-toluenesulphonate **2.3** from 2-phenoxyethanol **2.2** to give crude 3-phenoxyprop-1-yl *p*-toluenesulphonate **2.25** (0.26 g, 43%).

1-Iodo-3-phenoxypropane (**2.26**)

Crude 3-phenoxyprop-1-yl *p*-toluenesulphonate **2.25** (0.1 g, 0.3 mmol) was refluxed with sodium iodide (73 mg, 0.49 mmol) in acetone/dioxane (3:1) (2 ml) in an oil bath at 120 °C for 3 hours. The reaction mixture was evaporated to dryness under a stream of argon and the residue chromatographed over silica gel with hexane/$CHCl_3$ (9:1) to give pure 1-iodo-3-phenoxypropane **2.26**. ^{1}H NMR (200 MHz) δ: 2.32 (m, 2H), 3.4 (t, J = 8 Hz, 2H), 4.1 (t, J = 8 Hz, 2H), 6.9-7.4 (m, 5H, aromatic protons).

2-Phenylethyl p-*toluenesulphonate* (**2.29**)

The precursor for the tosylate **2.29**, 2-phenylethyl alcohol **2.28**, was prepared from 1-bromo-2-phenylethane **2.27** (0.5 g, 2.7 mmol) according to the procedure described for the preparation of 2-phenoxyethanol **2.2** from β-bromophenetole **2.1**. The target tosylate **2.29** was prepared from crude **2.28** (0.2 g, 1.6 mmol) according to the procedure described for the preparation of 2-phenoxyethyl *p*-toluenesulphonate **2.3** from 2-phenoxyethanol **2.2** to give crude 2-phenylethyl *p*-toluenesulphonate **2.29** (0.39 g, 86%).

1-Iodo-2-phenylethane (**2.30**)

Crude 2-phenylethyl *p*-toluenesulphonate **2.29** (0.1 g, 0.36 mmol) was refluxed with sodium iodide (80 mg, 0.53 mmol) in acetone/dioxane (1:1) (2 ml) in an oil bath at 120 °C for 1.5 hours. The reaction mixture was evaporated to dryness under a stream of argon and the residue chromatographed over silica gel with hexane/$CHCl_3$ (9:1) to give pure 1-iodo-2-phenylethane **2.30**. ^{1}H NMR (200 MHz) δ: 3.2 (t, J = 8 Hz, 2H), 3.4 (t, J = 8 Hz, 2H), 7.2-7.4 (m, 5H, aromatic protons).

6.3.2 Synthesis of the Benzamide Derivatives

2-Hydroxy-3-methoxy-N-*(2-piperidin-1-ylethyl)benzamide* (**2.33**)

Thionyl chloride (0.75 ml, 10.3 mmol) and a few drops of dimethylformamide (20 µl) were added to a solution of 3-methoxysalicylic acid **2.31** (0.5 g, 2.97 mmol) in chloroform (5 ml). The mixture was refluxed for 30 min., after which some additional thionyl chloride (0.5 ml) and dimethylformamide (50 µl) were added. Heating was continued for another 30 min., after which the solvent and excess thionyl chloride were distilled off on a water bath at 80°C under reduced pressure. The residue was dissolved in chloroform (2.5 ml) and added to a solution of 1-(2-aminoethyl)piperidine (0.42 g, 3.3 mmol) in chloroform (1.0 ml). Triethylamine (1.9 ml) was added to this mixture, which was stirred overnight at room temperature, diluted with chloroform (10 ml) and washed successively with 2% sodium hydrogen carbonate (10 ml) and water (10 ml). The solution of the organic product in chloroform was dried over anhydrous $MgSO_4$, the drying agent filtered off, and the filtrate evaporated to dryness at 80°C under reduced pressure. The resulting crude residue was purified by column chromatography over silica gel, using diisopropyl ether/methanol/aqueous ammonia (80:20:1) as mobile phase to give 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33** as a light brown oil (315 mg, 38%). ^{1}H NMR (300 MHz) δ: 1.43-1.51 (m, 2H, piperidine CH_2), 1.58-1.66 (m, 4H, piperidine CH_2), 2.48-2.51 (m, 4H, piperidine CH_2), 2.58-2.62 (t, J = 6.0 Hz, 2H, NCH_2), 3.52-3.57 (m, 2H, CONHCH_2), 3.9 (s, 3H, OCH_3), 6.79-6.84 (t, J = 8.1 Hz, 1H, aromatic proton), 6.97-7.01(d, J = 7.9 Hz, 1H, aromatic proton), 7.10-7.13 (d, J = 8.2 Hz, 1H, aromatic proton),

7.70 (bs, 1H, N*H*). ^{13}C NMR (75 MHz) δ: 24.27 (piperidine CH_2), 25.97 (piperidine CH_2), 35.99 ($CONH\underline{C}H_2CH_2$), 54.40 (piperidine NCH_2), 56.25 ($CONHCH_2\underline{C}H_2$), 56.91 (OCH_3), 115.02 (aromatic C), 115.33 (aromatic C), 117.91 (aromatic C), 118.37 (aromatic C), 149.62 (aromatic C), 152.16 (aromatic C-OH), 170.30 (C=O). MS (EI) *m/z* (%) 278 M^+ (5), 151 (6), 111 (12), 98 (100), 41 (6), 28 (5).

*3-Methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)ethoxy]benzamide* (**2.41**)

A mixture of ethylene glycol **2.34** (dried by distillation over anhydrous sodium sulphate) (810 mg, 13.0 mmol) and *p*-toluenesulphonyl chloride (5.7 g, 30 mmol) was dissolved in dry dichloromethane (30 ml). Triethylamine (4.5 ml) was added to the mixture, the mixture was heated under reflux for 6 hours, cooled, the triethylammonium chloride filtered off, and the filter cake washed with dichloromethane. The combined filtrate and washings was evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel (20 g), using chloroform/hexane (70:30), followed by chloroform as mobile phases to give a pure fraction of the coupling reagent ethylene di-*p*-toluenesulphonate (TET) **2.35**. A 20% aqueous solution of tetrabutylammonium hydroxide (0.5 ml, 0.38 mmol) was added to a solution of 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33** (80 mg, 0.29 mmol) in acetonitrile (15 ml). The solution was concentrated to dryness under reduced pressure at 50°-55°C. The residue was re-dissolved in acetonitrile (8 ml) and the solution was again concentrated to dryness as before. This step was repeated twice, using dry acetonitrile, and a final temperature of 65°-70°C to ensure complete dryness of the tetrabutylammonium phenoxide. The dried material was dissolved in dry acetonitrile (3 ml) and treated with a solution of TET **2.35** (1.0 g, 2.7 mmol) in dry acetonitrile (6 ml). The mixture was refluxed for approximately 1.5 hours until TLC (Method 4) indicated total consumption of the benzamide **2.33**. The reaction mixture was concentrated to dryness, after which the residue was re-dissolved in chloroform (10 ml), the chloroform solution washed once with water (5 ml), dried over anhydrous sodium sulphate, and the drying agent filtered off. The filtrate was concentrated to dryness, re-dissolved in acetonitrile (3 ml) under gentle heating, and the solution was cooled overnight at -10°C to allow as much as possible of the excess TET to

crystallise. The crystals were filtered off, washed with small portions of chilled acetonitrile, and the combined filtrate and washings were concentrated to dryness. The residue was chromatographed over silica gel (4 g) with chloroform as mobile phase to remove the remainder of the TET as well as other impurities. The required product was eluted with diisopropyl ether/methanol/triethylamine (80:20:2) to give crude **2.41** (219 mg). Further column chromatography over silica gel and elution with diisopropyl ether/methanol/aqueous ammonia (85:15:0.5) gave pure 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)ethoxy]benzamide **2.41** (78 mg, 57%). ^{1}H NMR (300 MHz) δ: 1.41-1.45 (m, 2H, piperidine C*H*$_2$), 1.51-1.59 (m, 4H, piperidine C*H*$_2$), 2.41-2.45 (s, m, 3H, 4H, Ts-C*H*$_3$ and piperidine C*H*$_2$ overlapping), 2.51-2.55 (t, *J* = 6.4 Hz, 2H, NC*H*$_2$), 3.54-3.60 (m, 2H, CONHC*H*$_2$), 3.86 (s, 3H, OC*H*$_3$), 4.29- 4.40 (m, AA'BB' spin system, 4H, OC*H*$_2$C*H*$_2$), 7.02-7.05 (dd, $^3J_{vic}$ = 8.2 Hz, 1H, phenyl *H*), 7.14-7.20 (t, *J* = 8.0 Hz, 1H, phenyl *H*), 7.36-7.38, 7.81-7.84 (m, AA'BB' spin system, 2H, 2H, Ts aromatic *H*), 7.67-7.70 (dd, $^3J_{vic}$ = 8.0 Hz, 1H, phenyl *H*), 8.04 (bs, 1H, N*H*). ^{13}C NMR (75 MHz) δ: 21.74 (Ts-C$\underline{H}_3$), 24.52 (piperidine CH_2), 26.08 (piperidine CH_2), 36.88 (CONHC$\underline{H}_2$CH$_2$), 54.57 (piperidine NCH$_2$), 56.27 (CONHCH$_2$C$\underline{H}_2$), 57.69 (OCH$_3$), 68.77 (C$\underline{H}_2$OTs), 70.94 (OCH$_2$), 115.49 (phenyl C), 123.35 (phenyl C), 125.02 (phenyl C), 128.05 (phenyl C), 128.40 (Ts aromatic C), 130.36 (Ts aromatic C), 133.31 (Ts aromatic C), 145.51 (Ts aromatic C), 145.78 (phenyl C), 152.73 (phenyl C), 165.42 (C=O). MS (EI) *m/z* (%) 476 M^+ (0.1), 366 (1), 276 (2), 291 (27), 235 (23), 177 (22), 149 (13), 98 (100), 57 (87), 28 (92).

2-(2-Iodoethoxy)-3-methoxy-N-*(2-piperidin-1-ylethyl)benzamide* (**2.42**)

The starting compound 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)-ethoxy]benzamide **2.41** (20 mg, 0.042 mmol) was dissolved in acetone (0.5 ml) containing sodium iodide (8 mg, 0.053 mmol) and the mixture was heated at reflux temperature in a sealed vial (5 ml) on a hot plate for approximately 1 hour. The reaction mixture was subsequently chromatographed over silica gel (3 g) and the required product was eluted with diisopropyl ether/methanol/aqueous ammonia (80:20:0.2) to give pure 2-(2-iodoethoxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.42** (14 mg, 77%). ^{1}H NMR (300 MHz) δ: 1.46-1.54 (m, 2H,

piperidine CH_2), 1.63-1.70 (m, 4H, piperidine CH_2), 2.52-2.55 (m, 4H, piperidine CH_2), 2.63-2.67 (t, J = 6.2 Hz, 2H, NCH_2), 3.48-3.53 (t, J = 7.3 Hz, 2H, CH_2I), 3.63-3.70 (m, 2H, $CONHCH_2$), 3.91 (s, 3H, OCH_3), 4.34-4.39 (t, J = 7.3 Hz, 2H, $ArOCH_2$), 7.06-7.09 (dd, $^3J_{vic}$ = 8.2 Hz, 1H, aromatic *H*), 7.17-7.22 (t, J = 8.1 Hz, 1H, aromatic *H*), 7.70-7.73 (dd, $^3J_{vic}$ = 8.0 Hz, ^4J = 1.6 Hz, 1H, aromatic *H*), 8.23 (bs, 1H, N*H*). ^{13}C NMR (75 MHz) δ: 1.80 (CH_2I), 24.20 (piperidine CH_2), 25.75 (piperidine CH_2), 36.68 ($CONHCH_2CH_2$), 54.66 (piperidine NCH_2), 56.34 ($CONHCH_2CH_2$), 57.72 (OCH_3), 74.24 (OCH_2), 115.59 (aromatic C), 123.25 (aromatic C), 125.01 (aromatic C), 127.80 (aromatic C), 145.94 (aromatic C), 152.76 (aromatic C), 165.76 (C=O). MS (EI) *m/z* (%) 432 M^+ (0.2), 322 (2), 276 (0.8), 220 (0.8), 193 (1), 150 (4), 111 (24), 98 (100), 55 (3), 41 (2).

3-Methoxy-N-*(2-piperidin-1-ylethyl)-2-[1-(*p-*toluenesulphonyloxy)prop-2-yloxy]benzamide* (**2.43**) and *3-methoxy*-N-*(2-piperidin-1-ylethyl)-2-[2-(*p-*toluenesulphonyloxy)prop-1-yloxy]benzamide* (**2.45**)

Triethylamine (8.0 ml) was added to a mixture of propane-1,2-diol (2.0 g, 26.3 mmol) and *p*-toluenesulphonyl chloride (11.0 g, 57.7 mmol) in dry dichloromethane (60 ml), and the solution was refluxed in an oil bath at a temperature varying between 80°-120°C for a total of 9 hours. The reaction mixture was cooled, filtered, and the filter cake washed with a small portion of dichloromethane. The combined filtrate and washing was evaporated to dryness under reduced pressure and the residue chromatographed over silica gel (30 g), using chloroform/hexane (50:50) as mobile phase, to give a pure fraction of the coupling reagent propylene 1,2-di-*p*-toluenesulphonate (TPT) **2.37**. A 20% aqueous solution of tetrabutylammonium hydroxide (0.5 ml, 0.38 mmol) was added to a solution of 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33** (75 mg, 0.27 mmol) in acetonitrile (14 ml). The solution was concentrated to dryness under reduced pressure at 40-50°C. The residue was dried by repeated dissolution in acetonitrile (7 ml) and concentration to dryness, after which the residue was dissolved in dry acetonitrile (3 ml) and treated with a solution of TPT **2.37** (1.0 g, 2.6 mmol) in dry acetonitrile (4.5 ml). The reaction mixture was heated under reflux for 30 min. until TLC (Method 4 or 5) indicated total consumption of the benzamide **2.33**. The

reaction mixture was concentrated to dryness, and the residue was chromatographed over silica gel (6 g). Chloroform was used as mobile phase to remove apolar impurities, followed by diisopropyl ether/methanol/aqueous ammonia [85:15:0.5] (75 ml) and [75:25:0.2] (25 ml) to isolate the mixture of isomers. The crude isolated material was subjected to further chromatography over fresh silica gel (6g), using diisopropyl ether/isopropanol/aqueous ammonia [85:15:0.5] (125 ml), followed by diisopropyl ether/methanol/aqueous ammonia [85:15:0.5] (25 ml) as mobile phases. Four main fractions were collected. The first fraction consisted mainly of 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[1-(*p*-toluenesulphonyloxy)prop-2-yloxy]benzamide **2.43** (25 mg, 19%). ^{1}H NMR (600MHz) δ: 1.31 (d, J = 6.4 Hz, 3H, CHC*H*$_3$), 1.42-1.46 (m, 2H, piperidine C*H*$_2$), 1.54-1.57 (m, 4H, piperidine C*H*$_2$), 2.42-2.45 (s, m, 3H, 4H, Ts-C*H*$_3$ and piperidine C*H*$_2$ overlapping), 2.50-2.52 (t, J = 6.1 Hz, 2H, NC*H*$_2$), 3.49-3.59 (m, 2H, CONHC*H*$_2$), 3.84 (s, 3H, OC*H*$_3$), 4.16 (AB spin system, J_{gem} = 10.5 Hz, J_{vic} = 4.4 Hz, 1H, C*H*$_2$OTs), 4.27 (AB spin system, J_{gem} = 10.5 Hz, J_{vic} = 5.2 Hz, 1H, C*H*$_2$OTs), 4.70-4.73 (m, 1H, OC*H*CH$_3$), 7.0 (dd, $^3J_{vic}$ = 8.2 Hz, 1H, phenyl *H*), 7.11-7.14 (t, J = 7.9 Hz, 1H, phenyl *H*), 7.32-7.34, 7.75-7.77 (m, AA'BB' spin system, 4H, Ts aromatic *H*), 7.64-7.66 (dd, $^3J_{vic}$ = 7.9 Hz, 1H, phenyl *H*), 7.93 (bs, 1H, N*H*). ^{13}C NMR (150 MHz) δ: 16.89 (CH<u>C</u>H$_3$, 21.91 (Ts-<u>C</u>H$_3$), 24.67 (piperidine CH$_2$), 26.09 (piperidine CH$_2$), 36.88 (CONH<u>C</u>H$_2$CH$_2$), 54.67 (piperidine NCH$_2$), 56.29 (CONHCH$_2$<u>C</u>H$_2$), 57.80 (OCH$_3$), 72.05 (<u>C</u>H$_2$OTs), 76.53 (CH-O), 115.25 (phenyl C), 123.19 (phenyl C), 124.49 (phenyl C), 128.17 (phenyl C), 128.59 (Ts aromatic C), 130.11 (Ts aromatic C), 133.17 (Ts aromatic C), 144.29 (Ts aromatic C), 145.14 (phenyl C), 152.43 (phenyl C), 165.43 (C=O). MS (EI) *m/z* (%) 490 M$^+$ (0.1), 380 (1), 363 (0.5), 318 (0.5), 276 (3), 220 (1), 151 (3), 111 (13), 98 (100), 70 (4), 41 (3). The second fraction consisted of a mixture of isomers enriched in **2.43** (32 mg, 24%); the third fraction consisted of a mixture of isomers enriched in **2.45** (20 mg, 15%); the fourth fraction consisted mainly of 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)prop-1-yloxy]benzamide **2.45** (18 mg, 14%). ^{1}H NMR (600MHz) δ: 1.43 (d, m, J = 6.6 Hz, 3H, 2H, CHC*H*$_3$ and piperidine C*H*$_2$ overlapping), 1.56-1.60 (m, 4H, piperidine C*H*$_2$), 2.42 (s, 3H, Ts-C*H*$_3$), 2.45 (m, 4H, piperidine C*H*$_2$), 2.54-2.56 (t, 6.3 Hz, 2H, NC*H*$_2$), 3.49-3.63 (m, 2H, CONHC*H*$_2$), 3.84 (s, 3H, OC*H*$_3$), 3.95 (dd, J_{gem} = 10.5 Hz, J_{vic} = 4.0 Hz, 1H, CHC*H*$_2$O), 4.18

(dd, J_{gem} = 10.5 Hz, J_{vic} = 5.7 Hz, 1H, CHC*H*$_2$O), 4.98-5.03 (m, 1H, C*H*OTs), 7.0 (dd, $^3J_{vic}$ = 8.1 Hz, 1H, phenyl *H*), 7.12-7.15 (t, *J* = 8.0 Hz, 1H, phenyl *H*), 7.30-7.31, 7.80-7.82 (m, AA'BB' spin system, 2H, 2H, Ts aromatic *H*), 7.62-7.64 (dd, $^3J_{vic}$ = 7.9 Hz, 1H, phenyl *H*), 7.87 (bs, 1H, N*H*). ^{13}C NMR (150 MHz) δ: 17.75 (CH$\underline{C}$H$_3$), 21.88 (Ts-$\underline{C}$H$_3$), 24.63 (piperidine CH$_2$), 26.13 (piperidine CH$_2$), 37.05 (CONH$\underline{C}$H$_2$CH$_2$), 54.68 (piperidine NCH$_2$), 56.31 (CONHCH$_2\underline{C}$H$_2$), 57.84 (OCH$_3$), 75.14 ($\underline{C}$H-OTs), 77.85 (OCH$_2$), 115.34 (phenyl C), 123.10 (phenyl C), 124.73 (phenyl C), 127.78 (phenyl C), 128.06 (Ts aromatic C), 130.01 (Ts aromatic C), 134.43 (Ts aromatic C), 144.99 (Ts aromatic C), 145.80 (phenyl C), 152.38 (phenyl C), 165.30 (C=O). MS (EI) *m/z* (%) 490 M^+ (0.2), 380 (3), 361 (2), 295 (7), 291 (35), 251 (9), 177 (17), 137 (11), 111 (32), 98 (100), 55 (4), 41 (7).

2-(1-Iodoprop-2-yloxy)-3-methoxy-N-*(2-piperidin-1-ylethyl)benzamide* (**2.44**).

This compound was prepared from 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[1-(*p*-toluenesulphonyloxy)prop-2-yloxy]benzamide **2.43** (17 mg, 0.035 mmol) according to the procedure used for the preparation of 2-(2-iodoethoxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.42**. Chromatographic purification of the product gave 8 mg (52%) of the target compound 2-(1-iodoprop-2-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.44**. ^{1}H NMR (600MHz) δ: 1.45-1.49 (d, m, *J* = 6.2 Hz, CHC*H*$_3$ and piperidine C*H*$_2$ overlapping, 3H, 2H), 1.59-1.63 (m, piperidine C*H*$_2$, 4H), 2.44 (m, piperidine C*H*$_2$, 4H), 2.54-2.56 (t, *J* = 6.0 Hz, NC*H*$_2$, 2H), 3.44-3.47 (AB spin system, C*H*$_2$I, J_{gem} = 9.9 Hz, J_{vic} = 7.6 Hz, 1H), 3.49-3.51 (AB spin system, C*H*$_2$I, J_{gem} = 9.9 Hz, J_{vic} = 4.4 Hz, 1H), 3.53-3.66 (m, CONHC*H*$_2$, 2H), 3.9 (s, OC*H*$_3$, 3H), 4.52-4.55 (m, OC*H*CH$_3$, 1H), 7.03 (dd, $^3J_{vic}$ = 8.2 Hz, phenyl *H*, 1H), 7.14-7.17 (t, *J* = 8.0 Hz, phenyl *H*, 1H), 7.69 (dd, $^3J_{vic}$ = 7.8 Hz, phenyl *H*, 1H), 8.03 (bs, N*H*, 1H). ^{13}C NMR (150 MHz) δ: 9.69 (CH$_2$I), 19.99 (OCH$\underline{C}$H$_3$), 24.41 (piperidine CH$_2$), 25.88 (piperidine CH$_2$), 36.70 (CONH$\underline{C}$H$_2$CH$_2$), 54.55 (piperidine NCH$_2$), 56.08 (CONHCH$_2\underline{C}$H$_2$), 57.82 (OCH$_3$), 78.59 (O$\underline{C}$HCH$_3$), 115.03 (phenyl C), 122.93 (phenyl C), 124.28 (phenyl C), 128.46 (phenyl C), 144.15 (phenyl C), 152.30 (phenyl C), 165.33 (C=O). MS (EI) *m/z* (%) 336 (1), 278 (1), 259 (0.5), 179 (2), 142 (5), 111 (20), 98 (89), 55 (9), 18 (100).

2-(2-Iodoprop-1-yloxy)-3-methoxy-N-*(2-piperidin-1-ylethyl)benzamide* (**2.46**).

This compound was prepared from 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)prop-1-yloxy]benzamide **2.45** (14 mg, 0.028 mmol) according to the procedure used for the preparation of 2-(2-iodoethoxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.42**. Chromatographic purification of the product gave 8 mg (63%) of the target compound 2-(2-iodoprop-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.46**. ^{1}H NMR (600MHz) δ: 1.45-1.47 (m, piperidine C*H*$_2$, 2H), 1.58-1.61 (m, piperidine C*H*$_2$, 4H), 2.02 (d, J = 6.7Hz, I-CHC*H*$_3$, 3H), 2.45 (m, piperidine C*H*$_2$, 4H), 2.57 (br t, NC*H*$_2$, 2H), 3.59-3.62 (m, CONHC*H*$_2$, 2H), 3.88 (s, OC*H*$_3$, 3H), 4.12 (dd, OC*H*$_2$CH, J_{gem} = 10.3 Hz, J_{vic} = 7.5 Hz, 1H), 4.26 (dd, OC*H*$_2$CH, J_{gem} = 10.3 Hz, J_{vic} = 5.9 Hz, 1H), 4.47-4.53 (m, IC*H*CH$_3$, 1H), 7.02 (dd, $^3J_{vic}$ = 8.1 Hz, phenyl *H*, 1H), 7.13-7.15 (t, J = 8.0 Hz, phenyl *H*, 1H), 7.64 (dd, $^3J_{vic}$ = 8.0 Hz, phenyl *H*, 1H), 8.0 (bs, N*H*, 1H). ^{13}C NMR (150 MHz) δ: 23.54 (CH-I), 24.62 (piperidine CH$_2$), 25.11 (ICH$\underline{C}$H$_3$), 26.13 (piperidine CH$_2$), 37.04 (CONH$\underline{C}$H$_2$CH$_2$), 54.80 (piperidine NCH$_2$), 56.37 (CONHCH$_2\underline{C}$H$_2$), 58.01 (OCH$_3$), 80.28 (OCH$_2$), 115.35 (phenyl C), 123.10 (phenyl C), 124.78 (phenyl C), 127.87 (phenyl C), 145.90 (phenyl C), 152.48 (phenyl C), 165.48 (C=O). MS (EI) *m/z* (%) 336 (0.5), 278 (1.0), 254 (1.0), 193 (0.5), 151 (3), 111 (23), 98 (100), 55 (6), 18 (18).

(E)-*3-(Tributylstannyl)prop-2-en-1-yl p-toluenesulphonate (TBS-PROP-TOS)* (**2.40**)

This compound was synthesized from propargyl alcohol **2.38** in a two-step procedure consisting of the addition of tributyltin hydride to the alkynyl group, followed by the tosylation of the hydroxyl group with *p*-toluenesulphonyl chloride (*p*-TsCl) according to the protocol of Musachio and Lever (1992). Thus, a mixture of propargyl alcohol **2.38** (0.4 g, 7.1 mmol), tri-butyltin hydride (2.7 g, 9.3 mmol) and a catalytic amount of 2,2'-azobisisobutyronitrile (15 mg) was heated in an oil bath (80°C) for 2.5 hours. The product mixture was chromatographed over silica gel (15 g), using hexane/ethyl acetate (92:8) as mobile phase. The fraction enriched in the target compound, (*E*)-3-(tributylstannyl)prop-2-en-1-ol **2.39a** (the more polar component on TLC), was subjected to further chromatographic purification over silica gel (20 g), using hexane/ethyl acetate (92:8), followed by (96:4) as mobile phases. The purified product **2.39a**

(366 mg, 1.1 mmol) was reacted with *p*-TsCl (266 mg, 1.4 mmol) and potassium trimethylsilanolate (650 mg, 5.1 mmol) in diethyl ether at -20°C for approximately 2 hours. After work-up, the crude product was chromatographed three times over silica gel (6 g) with mixtures of hexane/ethyl acetate (95:5, 85:15, and finally 98:2) to give TBS-PROP-TOS **2.40** as a colourless oil (187 mg, 35%). The ^{1}H NMR data are given in Table 2.1.

3-Methoxy-N-*(2-piperidin-1-ylethyl)-2-[3-(tributylstannyl)prop-2-en-1-yloxy]benzamide* (**2.47**)
A 20% aqueous solution of tetrabutylammonium hydroxide (0.2 ml, 0.16 mmol) was added to a solution of 2-hydroxy-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.33** (30 mg, 0.11 mmol) in acetonitrile (3 ml). The solution was concentrated to dryness under reduced pressure at 40-50°C. To ensure complete removal of any residual water, the residue was dissolved in dry acetonitrile (3 ml), the solution concentrated to dryness, and this procedure was repeated twice more. The dried residue was dissolved in dry acetonitrile (1.2 ml) and treated with a solution of TBS-PROP-TOS **2.40** (76 mg, 0.15 mmol) in dry acetonitrile (1.0 ml). This reaction mixture was heated and stirred in an oil bath at a temperature of 100-110°C until TLC analysis (Method 4) indicated total consumption of the starting compound **2.33**. The reaction mixture was subsequently concentrated with a stream of nitrogen, the residue was diluted with ethyl acetate, and chromatographed over silica gel (4 g) with hexane/ethyl acetate (90:10) and finally with diisopropyl ether/methanol/aqueous ammonia (80:20:0.5) to give pure 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[3-(tributylstannyl)prop-2-en-1-yloxy]benzamide **2.47** (55 mg, 84%). ^{1}H NMR (300 MHz) δ: 0.85-0.93 (m, *n*-Bu protons, 15H), 1.23-1.36 (m, Bu protons, 6H), 1.41-1.50 (m, Bu protons and piperidine C*H*$_2$ overlapping, 8H), 1.56-1.63 (m, piperidine C*H*$_2$, 4H), 2.43-2.47 (m, piperidine C*H*$_2$, 4H), 2.53-2.57 (t, J = 6.4 Hz, NC*H*$_2$, 2H), 3.55-3.61 (m, CONHC*H*$_2$, 2H), 3.9 (s, OC*H*$_3$, 3H), 4.62-4.65 (dd, ArOC*H*$_2$, $^3J_{vic}$ = 7.0 Hz, ^4J = 2.7 Hz, 2H), 6.24-6.28 (m, C*H*=C*H* overlapping, 2H), 7.03-7.06 (dd, $^3J_{vic}$ = 8.2 Hz, phenyl *H*, 1H), 7.13-7.18 (t, J = 8.0 Hz, phenyl *H*, 1H), 7.72-7.75 (dd, $^3J_{vic}$ = 8.0 Hz, phenyl *H*, 1H), 8.39 (bs, N*H*, 1H). ^{13}C NMR (75 MHz) δ: 9.18 (Bu_3-C), 13.43 (Bu_3-C), 24.18 (piperidine CH_2), 25.75 (piperidine CH_2), 27.07 (Bu_3-C), 28.83 (Bu_3-C), 36.61 (CONH$\underline{C}H_2CH_2$), 54.33 (piperidine NCH_2), 55.89 (CONHCH$_2\underline{C}H_2$), 57.47 (OCH_3), 77.42 (OCH_2), 114.99 (phenyl C), 122.93

(phenyl C), 124.20 (phenyl C), 127.74 (phenyl C), 134.25 (CH=CH-Sn), 142.95 (CH=CH), 146.27 (phenyl C), 152.84 (phenyl C), 165.46 (C=O). MS (EI) *m/z* (%) 607 M^+ (0.2), 551 (19), 498 (3), 397 (10), 378 (2), 268 (2), 177 (5), 155 (64), 98 (100), 41 (5).

2-(3-Iodoprop-2-en-1-yloxy)-3-methoxy-N-*(2-piperidin-1-ylethyl)benzamide* (**2.48**)

A solution of I_2 (9 mg, 0.04 mmol) in dichloromethane (0.57 ml) was added to a solution of 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[3-(tributylstannyl)prop-2-en-1-yloxy]benzamide **2.47** (20 mg, 0.03 mmol) in dichloromethane (0.1 ml). The reaction mixture was stirred at room temperature for 1 hour, after which the reaction was quenched by the addition of $Na_2S_2O_5$ (12.8 mg, 0.06 mmol) in water (1.5 ml). The aqueous phase was made alkaline with 1N NaOH (0.1 ml), additional dichloromethane (1 ml) was added to the product, and the layers were separated. The aqueous phase was extracted twice with dichloromethane (0.5 ml), the combined extracts were dried over anhydrous $MgSO_4$, the drying agent filtered off, and the filtrate concentrated with a stream of nitrogen. The crude residue was chromatographed over silica gel (4 g) with diisopropyl ether/methanol/aqueous ammonia (90:10:1) to give pure 2-(3-iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.48** (10.7 mg, 73%). ^{1}H NMR (300 MHz) δ: 1.44-1.50 (m, piperidine C*H*$_2$, 2H), 1.57-1.64 (m, piperidine C*H*$_2$, 4H), 2.44-2.47 (m, piperidine C*H*$_2$, 4H), 2.53-2.57 (t, J = 6.2 Hz, NC*H*$_2$, 2H), 3.56-3.62 (m, CONHC*H*$_2$, 2H), 3.9 (s, OC*H*$_3$, 3H), 4.47-4.53 (dd, ArOC*H*$_2$, $^3J_{vic}$ = 6.4 Hz, ^4J = 1.3 Hz, 2H), 6.52-6.58 (dt, $^3J_{vic}$ = 14.6 Hz, ^4J = 1.4 Hz, CH=C*H*I, 1H), 6.83-6.93 (dt, $^3J_{vic}$ = 14.6 Hz, ^4J = 6.4 Hz, C*H*=CHI, 1H), 7.05-7.08 (dd, $^3J_{vic}$ = 8.2 Hz, phenyl *H*, 1H), 7.17-7.22 (t, J = 8.1 Hz, phenyl *H*, 1H), 7.69-7.72 (dd, $^3J_{vic}$ = 7.9 Hz, phenyl *H*, 1H), 8.2 (bs, N*H*, 1H). ^{13}C NMR (150 MHz) δ: 24.49 (piperidine CH_2), 26.06 (piperidine CH_2), 36.79 (CONH$\underline{C}H_2CH_2$), 54.49 (piperidine NCH_2), 56.19 (CONHCH$_2\underline{C}H_2$), 57.44 (OCH_3), 75.33 (OCH_2), 80.94 (CH=CH-I), 115.01 (phenyl C), 122.88 (phenyl C), 124.72 (phenyl C), 127.89 (phenyl C), 141.13 (CH=CH-I), 145.53 (phenyl C), 152.52 (phenyl C), 165.12 (C=O). MS (EI) *m/z* (%) 444 M^+ (0.3), 334 (2), 194 (1), 167 (5), 111 (34), 98 (100), 55 (3), 42 (2).

6.3.3 Synthesis of the Heterocyclic Amine Derivatives

6.3.3.1 Synthesis of the alkylating agents and the benzotriazole derivatives

2-Hydroxyethyl benzoate (**2.50**)

Ethylene glycol was dried by distillation over anhydrous sodium sulphate. Concentrated sulphuric acid (0.5 ml) and molecular sieves (3Å, 5 g) were added to a mixture of benzoic acid **2.49** (13.0 g, 0.1 mole) and dry ethylene glycol **2.34** (19.6 g, 0.3 mole). The mixture was suspended in an oil bath (170°-185°C) and refluxed for 3 hours, diluted with water (100 ml), and repeatedly extracted with chloroform (50 ml). The resulting emulsion was filtered through a pad of filter aid, and the organic phase was separated from the supernatant water layer. The combined chloroform extracts were washed with water (50 ml) and sodium hydrogen carbonate (6%, 40 ml), dried over anhydrous $MgSO_4$, the drying agent was filtered off, and the filtrate evaporated to dryness to give 2-hydroxyethyl benzoate **2.50** as an oily product that solidified into a soft wax upon cooling (12.8 g, 72%). ^{1}H NMR (300 MHz) δ: 2.76 (bs, O*H*, 1H), 3.95-3.98 (t, J = 4.7 Hz, CH_2OH, 2H), 4.46-4.49 (t, J = 4.6 Hz, CO_2CH_2, 2H), 7.44-7.50 (m, *H*-3, *H*-5 phenyl, 2H), 7.57- 7.63 [t (with fine structure), *H*-4 phenyl, 1H], 8.08-8.12 (m, *H*-2, *H*-6 phenyl, 2H). ^{13}C NMR (75 MHz) δ: 61.25 (CH_2OH), 66.56 ($CO\underline{C}H_2$), 128.53 (phenyl C), 129.81 (phenyl C), 129.99 (phenyl C), 133.31 (phenyl C), 167.23 (C=O). MS (EI) *m/z* (%) 148 ($M^+ - H_2O$) (0.5), 135 (2), 123 (43), 105 (100), 77 (52), 51 (17), 18 (7).

2-Chloromethoxyethyl benzoate (**2.51**) [Guédin-Vuong and Nakatani (1986)]

A mixture of paraformaldehyde (165 mg, 5.5 mmol) and dichloromethane (3 ml) was placed in a 10 ml conical flask together with a magnetic stirrer bar, and cooled to -15°C by means of a liquid nitrogen-ethanol mixture. A stream of hydrogen chloride (generated by the addition of concentrated H_2SO_4 to a mixture of ammonium chloride and 32% HCl) was bubbled through a concentrated H_2SO_4 trap, and passed through the vigorously stirred mixture until a clear solution was formed. The flow of hydrogen chloride was maintained, the solution was kept at -15 to -10°C and was treated dropwise with a solution of 2-hydroxyethyl benzoate **2.50** (0.8 g,

4.8 mmol) in dichloromethane (1.5 ml). After completion of the addition of the benzoate solution, the reaction mixture was stirred for another 5 minutes at -10 to -12°C, after which the introduction of hydrogen chloride was stopped and the residual hydrogen chloride driven from the reaction mixture using a brisk flow of nitrogen. After two minutes, the cold reaction mixture was poured into a chilled suspension of anhydrous potassium carbonate (8 g) in dichloromethane (20 ml). The mixture was stirred for 10-15 minutes, the potassium carbonate filtered off, and the solvent evaporated from the filtrate at 20°C under reduced pressure to give a light yellow oil (0.95 g, 93%). This crude product was immediately refrigerated at -10°C and was used as soon as possible.

1-[(2-Benzoyloxyethoxy)methyl]benzotriazole (**2.63**)

A solution of freshly prepared 2-chloromethoxyethyl benzoate **2.51** (526 mg, 2.4 mmol) in dichloromethane (8 ml) was added to a mixture of benzotriazole **2.62** (232 mg, 1.9 mmol) and anhydrous potassium carbonate (820 mg, 5.9 mmol) in dichloromethane (15 ml). The mixture was stirred at room temperature for 6 hours, after which the potassium carbonate was filtered off and the filtrate evaporated at 50°C under reduced pressure. The crude product was chromatographed over silica gel (7 g), using petroleum ether/ethyl acetate (4:1) as mobile phase. Two fractions of the target compound **2.63** of 140 mg (24%) and 207 mg (35%) were isolated in purities of 94% and 85% respectively. ^{1}H NMR (600 MHz) δ: 3.85 (t, J = 4.6 Hz, OCH_2, 2H), 4.41 (t, J = 4.6 Hz, CH_2OCO, 2H), 6.09 (s, NCH_2O, 2H), 7.37-7.41 (m, *H*-3, *H*-5 phenyl, *H* benzotriazole overlapping, 3H), 7.47-7.49 (t, J = 7.6 Hz, *H*-4 phenyl, 1H), 7.53-7.55 (t, J = 7.4 Hz, *H* benzotriazole, 1H), 7.68 (d, J = 8.3 Hz, *H* benzotriazole, 1H), 7.87 (d, J = 7.6 Hz, *H*-2, *H*-6 phenyl, 2H), 8.08 (d, J = 8.3 Hz, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 63.13 ($CH_2\underline{C}H_2$), 67.05 ($\underline{C}H_2CH_2$), 77.12 (NCH_2), 109.87 (C benzotriazole), 120.07 (C benzotriazole), 124.44 (C benzotriazole), 128.03 (C benzotriazole), 128.29 (phenyl C), 129.57 (phenyl C), 129.66 (phenyl C), 132.63 (C benzotriazole), 133.01 (phenyl C), 146.47 (C benzotriazole), 166.27 (C=O). MS (EI) *m/z* (%) 298 $[M+H]^+$ (1), 209 (3), 197 (3), 181 (6), 167 (6), 149 (70), 105 (100), 77 (43), 51 (8).

1-[(2-Hydroxyethoxy)methyl]benzotriazole (**2.64**)

Concentrated (25%) ammonia (25 ml) was added to a solution of 1-[(2-benzoyloxyethoxy)methyl]benzotriazole **2.63** (306 mg, 1.0 mmol) in methanol (25 ml). The mixture was stirred for a few hours at room temperature until TLC (Method 6) indicated a total conversion of **2.63** to the hydroxyethoxymethylbenzotriazole **2.64**. The reaction mixture was concentrated under reduced pressure at 40-50°C to a volume of 20 ml, the remaining aqueous suspension was extracted twice with chloroform (2 × 20 ml), and the chloroform distilled off under reduced pressure to give a crude extract. This extract was dissolved in toluene (20 ml) and the toluene solution washed with water (2 × 10 ml), dried over anhydrous $MgSO_4$, the drying agent filtered off and the filtrate evaporated to dryness to give an oil. This product was chromatographed over silica gel (4 g) with ethyl acetate/petroleum ether (2:1) to give pure 1-[(2-hydroxyethoxy)methyl]benzotriazole **2.64** (19 mg, 10%). More of the target compound **2.64** was recovered from the combined water layers by extraction with chloroform (10 ml), followed by the normal work-up procedures to give **2.64** as a crude oily product (171 mg, 86%). ^{1}H NMR (600 MHz) δ: 3.64-3.70 (m, AA'BB' spin system, OC*H*$_2$C*H*$_2$OH, 4H), 6.07 (s, NC*H*$_2$O, 2H), 7.41-7.44 (t, *J* = 7.9 Hz, *H* benzotriazole, 1H), 7.54-7.56 (t, *J* = 7.6 Hz, *H* benzotriazole, 1H), 7.68-7.70 (d, *J* = 8.3 Hz, *H* benzotriazole, 1H), 8.08-8.10 (d, *J* = 8.5 Hz, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 61.42 (CH_2OH), 70.72 (O$\underline{C}H_2CH_2$), 77.25 (NCH_2), 109.73 (C-benzotriazole), 120.13 (C-benzotriazole), 124.52 (C-benzotriazole), 128.16 (C-benzotriazole), 132.74 (C-benzotriazole), 146.37 (C-benzotriazole). MS (EI) *m/z* (%) 193 M^+ (21), 163 (34), 132 (33), 120 (34), 105 (45), 91 (47), 77 (100), 51 (27), 45 (68), 29 (8).

*1-[(2-*p*-Toluenesulphonyloxyethoxy)methyl]benzotriazole* (**2.65**)

p-Toluenesulphonyl chloride (40 mg, 0.21 mmol) was added to a solution of crude 1-[(2-hydroxyethoxy)methyl]benzotriazole (**2.64**) (24 mg, approximately 0.12 mmol) in dry pyridine (0.25 ml). The reaction mixture was refrigerated overnight at 5°C, after which it was diluted with water (2 ml) and extracted three times with $CHCl_3$ (3 × 0.5 ml). The combined chloroform extracts were washed with 0.1N H_2SO_4 (1 ml) and water (1 ml) and dried over anhydrous $MgSO_4$. The drying agent was filtered off and the filtrate evaporated to dryness with a stream

of nitrogen. The crude extract was chromatographed over silica gel (3 g), using the following mobile phases: petroleum ether/ethyl acetate (3:1), (2:1) and (1:1). A pure fraction of 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65** was collected (27 mg, 62%). ^{1}H NMR (300 MHz) δ: 2.45 (s, Ts-C*H*$_3$, 3H), 3.68-3.71 (m, X_2 component of A_2X_2 spin system, CH_2OCH_2, 2H), 4.12-4.15 (m, A_2 component of A_2X_2 spin system, CH_2OTs, 2H), 6.0 (s, NCH_2O, 2H), 7.33-7.36, 7.74-7.78 (m, AA'BB' spin system, Ts aromatic *H*, 4H), 7.44-7.49 (ddd, $^3J_{vic}$ = 8.4 Hz, *H* benzotriazole, 1H), 7.56- 7.61 (ddd, $^3J_{vic}$ = 8.3 Hz, *H* benzotriazole, 1H), 7.66-7.70 (d, with fine structure, *H* benzotriazole, 1H), 8.11-8.15 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (75 MHz) δ: 21.48 (Ts-$\underline{C}H_3$), 66.71 ($\underline{C}H_2$OTs), 68.19 (O$\underline{C}H_2CH_2$), 76.98 (NCH_2), 109.89 (C-benzotriazole), 120.26 (C-benzotriazole), 124.70 (C-benzotriazole), 128.08 (Ts aromatic C), 128.37 (C-benzotriazole), 130.04 (Ts aromatic C), 132.84 (Ts aromatic C), 133.05 (C-benzotriazole), 145.22 (Ts aromatic C), 146.67 (C-benzotriazole). HRMS: *m/z* M^+ = 347.0939, calc. for $C_{16}H_{17}O_4N_3S$ = 347.0939. MS (EI) *m/z* (%) 347 M^+ (33), 228 (9), 192 (26), 156 (9), 155 (100), 120 (29), 91 (80), 65 (10).

1-[(2-Iodoethoxy)methyl]benzotriazole (**2.66b**)

A mixture of 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65** (20 mg, 0.06 mmol), sodium iodide (15 mg, 0.1 mmol) and acetone (0.5 ml), together with a magnetic stirrer bar, were sealed in a 5 ml glass vial with a cap. The mixture was heated and stirred on a hot plate at reflux temperature for 40 minutes. The reaction product was concentrated with a stream of nitrogen and chromatographed over silica gel (3 g) with petroleum ether/ethyl acetate (2:1) to give pure 1-[(2-iodoethoxy)methyl]benzotriazole **2.66b** (14 mg, 80%). ^{1}H NMR (300 MHz) δ: 3.08-3.12 (t, *J* = 6.5 Hz, CH_2I, 2H), 3.71-3.75 (t, *J* = 6.5 Hz, OCH_2CH_2 , 2H), 6.03 (s, NCH_2, 2H), 7.37-7.43 (ddd, $^3J_{vic}$ = 8.4 Hz, *H* benzotriazole, 1 H), 7.50-7.56 (ddd, $^3J_{vic}$ = 8.4 Hz, *H* benzotriazole, 1 H), 7.67-7.71 (d, with fine structure, *H* benzotriazole, 1H), 8.05-8.09 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (75 MHz) δ: 1.06 (CH_2I), 69.73 (O$\underline{C}H_2CH_2$), 76.72 (NCH_2), 110.01 (C-benzotriazole), 120.26 (C-benzotriazole), 124.68 (C-benzotriazole), 128.30 (C-benzotriazole), 132.87 (C-benzotriazole), 146.70 (C-benzotriazole). HRMS: *m/z* M^+

= 302.9874, calc. for $C_9H_{10}ON_3I$ = 302.9868. MS (EI) *m/z* (%) 303 M^+ (37), 275 (11), 245 (9), 231 (16), 176 (44), 155 (100), 132 (18), 120 (19), 77 (42), 65 (12), 18 (12).

2-Hydroxyprop-1-yl benzoate (**2.54**) from 70% 1-bromo-2-propanol **2.52**

A solution of sodium hydroxide (20%, 5 ml) was added to a suspension of benzoic acid (3 g) in ethanol (5 ml), the mixture was heated until the solid material dissolved, and the solution was evaporated to near dryness under reduced pressure at 70°C. To ensure complete removal of residual water, the residue was dissolved in absolute ethanol (30 ml), and the solution was again concentrated to dryness under reduced pressure. This procedure was repeated once more. The sodium benzoate was finally dried under vacuum at 100°C. A mixture of sodium benzoate (1.58 g, 11 mmol) and 1-bromo-2-propanol **2.52** (1.56 g, 11.2 mmol) in dimethylformamide (DMF) (40 ml) was stirred in an oil bath at 120-130°C until the solid material was dissolved. The resulting solution was further heated for approximately 1 hour at 130°C, after which another portion of 1-bromo-2-propanol (0.77 g, 5.5 mmol) was added to the solution. Heating was continued at 130-140°C for 40 minutes, after which the DMF was distilled off at 100°C under reduced pressure. The residue was suspended in $CHCl_3$ (30 ml), and the mixture was successively washed with water (15 ml), sodium hydrogen carbonate (6%, 15 ml) and finally with water (15 ml). The chloroform solution was dried over anhydrous $MgSO_4$, the drying agent was filtered off, and the filtrate evaporated to dryness at 80°C under reduced pressure to give a crude product that was chromatographed over silica gel (8 g). Mixtures of petroleum ether/ethyl acetate (5:1 and 2:1) were used as mobile phases. Pure fractions were combined to give 2-hydroxyprop-1-yl benzoate **2.54** (1.35 g, 68%) in a 65% isomeric purity [HPLC Method 3(a)]. [Characterization was only done on the purer 2-hydroxyprop-1-yl benzoate **2.54**, the synthesis of which is described later].

1-Bromo-2-propanol (**2.52**) ["isomer-free"]

Method 1: A solution of 2,2,6,6-tetramethylpiperidine (2.83 g, 20 mmol) in dry THF (10 ml) was added dropwise to a freshly prepared solution of ethylmagnesium bromide (20 mmol) in dry THF (50 ml) under a slow stream of nitrogen. The mixture was heated under reflux for 4

hours. A solution of propylene oxide (20 mmol) in dry THF (10 ml) was added dropwise to the reaction mixture cooled at -5°C in an ice/salt bath. The solution was stirred at 0°C for 2 hours, and then at room temperature for 18-20 hours. A saturated solution of ammonium chloride (30 ml) was then carefully added under cooling. Two layers were formed. The THF was distilled off at room temperature under reduced pressure, and the resulting aqueous solution was extracted three times with diethyl ether (3 × 25 ml). The ethereal extract was washed with saturated NH_4Cl and NaCl solutions and dried overnight over anhydrous sodium sulphate. The drying agent was filtered off and the solvent evaporated under reduced pressure. To avoid isomerization the residue was distilled at an air-bath temperature of 50°C in a Kugelrohr apparatus at a vacuum of 1×10^{-3} Torr and cooling of the distilled fractions with dry ice. A nearly colourless liquid was obtained (1.15 g, 41%). GC-MS *m/z* (%) 138 M^+ (0.5), 140 M^+ (0.5), 125 (10), 123 (10), 95 (3), 93 (3), 45 (100), 43 (20).

Method 2: An analogous preparation, using diisopropylamine instead of 2,2,6,6-tetramethylpiperidine, was also carried out. Starting with diisopropylamine (12.6 g, 125 mmol), 1-bromo-2-propanol **2.52** was obtained as a colourless liquid (7.5 g, 43%) in an isomeric purity of 99.7 % (GC Method 1).

2-Hydroxyprop-1-yl benzoate (**2.54**) from 99.7% isomerically pure 1-bromo-2-propanol **2.52**

A mixture of sodium benzoate (3.0 g, 20.8 mmol) and 1-bromo-2-propanol **2.52** (3.5 g, 25 mmol) in HMPA (30 ml) was stirred in an oil bath at 60°C for 2 hours. The reaction mixture was poured into an HCl solution (5%, 60 ml) and the mixture was extracted three times with diethyl ether (3 × 45 ml). The combined ethereal extracts were washed twice with water (2 × 25 ml), dried over anhydrous $MgSO_4$, the drying agent filtered off, and the filtrate evaporated to dryness under reduced pressure to give a crude product (3.5 g). This product was chromatographed over silica gel (20 g) as before. The fractions containing the product were combined, evaporated to dryness, dissolved in chloroform (20 ml), washed with $NaHCO_3$ solution (6%, 10 ml) to remove traces of benzoic acid, and finally with water (10 ml). After drying over anhydrous $MgSO_4$, followed by filtration of the drying agent and removal of the

solvent under reduced pressure at 70-80°C, 2-hydroxyprop-1-yl benzoate **2.54** (2.18 g, 58%) was obtained in a 97% isomeric purity [HPLC Method 3(a)]. ^{1}H NMR (600 MHz) δ: 1.28-1.30 (d, J = 6.3 Hz, CHC*H*$_3$, 3H), 2.40 (bs, O*H*, 1H), 4.17-4.22 (m, C*H*OH, -CO_2-C*H*H overlap, 2H), 4.33-4.35 (dd, J_{gem} = 10.7 Hz, J_{vic} = 2.9 Hz, -CO_2-CH*H*, 1H), 7.43-7.45 (t, with fine structure, *H*-3, *H*-5 phenyl, 2H), 7.55-7.58 (t, with fine structure, *H*-4 phenyl, 1H), 8.05-8.06 (d, with fine structure, *H*-2, *H*-6 phenyl, 2H). ^{13}C NMR (150 MHz) δ: 19.27 (CH$\underline{C}$H$_3$), 66.18 ($\underline{C}$HOH), 70.04 ($CO_2\underline{C}H_2$), 128.38 (phenyl C), 129.61 (phenyl C), 129.84 [phenyl C (*ipso*)], 133.13 (phenyl C), 166.65 (C=O). MS (EI) *m/z* (%) 180 M^+ (0.5), 136 (21), 123 (10), 105 (100), 92 (14), 77 (45), 51 (15), 45 (7).

2-Chloromethoxyprop-1-yl benzoate (**2.56**) from 65% isomerically pure 2-hydroxyprop-1-yl benzoate **2.54**

2-Chloromethoxyprop-1-yl benzoate **2.56** was prepared from 2-hydroxyprop-1-yl benzoate **2.54** (1.0 g, 5.55 mmol) and paraformaldehyde (0.19 g, 6.33 mmol) according to the procedure used for the preparation of 2-chloromethoxyethyl benzoate **2.51**. The target compound **2.56** was obtained as a light-yellow crude oil (1.26 g, 100%).

2-Chloromethoxyprop-1-yl benzoate (**2.56**) from 97% isomerically pure 2-hydroxyprop-1-yl benzoate **2.54**

2-Chloromethoxyprop-1-yl benzoate **2.56** was prepared from 2-hydroxyprop-1-yl benzoate **2.54** (1.0 g, 5.55 mmol) and paraformaldehyde (0.19 g, 6.33 mmol) according to the procedure used for the preparation of 2-chloromethoxyethyl benzoate **2.51**. The dichloromethane solution, containing the target compound **2.56**, was concentrated under reduced pressure at room temperature to a volume of 17 ml. The approximate solid content of this solution (1.2 g, 100%) was determined by evaporating a small portion to dryness and calculating the total mass by means of extrapolation. The solution was immediately refrigerated at -10°C.

1-[(1-Benzoyloxyprop-2-yloxy)methyl]benzotriazole (**2.67**) from isomerically impure 2-chloromethoxyprop-1-yl benzoate **2.56**

This compound was prepared from benzotriazole (200 mg, 1.7 mmol), anhydrous potassium carbonate (0.7 g) and freshly prepared isomerically impure 2-chloromethoxyprop-1-yl benzoate **2.56** (503 mg, 2.2 mmol) according to the procedure used for the preparation of 1-[(2-benzoyloxyethoxy)methyl]benzotriazole (**2.63**). The reaction product was chromatographed over silica gel (8 g) using petroleum ether/ethyl acetate mixtures (10:1, 5:1, 2:1) as mobile phases. Fractions containing the product were combined and evaporated to dryness to give 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67** as an oil (413 mg, 79%) in an isomeric purity of 76% [HPLC Method 2(b)]. [Characterization was only done on the purer 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67**, the synthesis of which is described below].

1-[(1-Benzoyloxyprop-2-yloxy)methyl]benzotriazole (**2.67**) from isomerically pure 2-chloromethoxyprop-1-yl benzoate **2.56**

This compound was prepared from benzotriazole (300 mg, 2.52 mmol), anhydrous potassium carbonate (1.05 g) and freshly prepared 2-chloromethoxyprop-1-yl benzoate **2.56** (700 mg, 3.1 mmol) according to the procedure used for the preparation of 1-[(2-benzoyloxyethoxy)methyl]benzotriazole (**2.63**). Chromatography was carried out over silica gel (25 g), using petroleum ether/ethyl acetate mixtures (6:1, 2:1, and 1:1) as mobile phases. The combined fractions containing the product gave a crude white solid (683 mg, 87% pure). Crystallisation of this material (615 mg) from methanol (3 ml) gave the pure target compound **2.67** (389 mg, 55%), in a purity of 99% [HPLC Method 2(b)], m.p. 68-68.5°C, ^{1}H NMR (600 MHz) δ: 1.20-1.21 (d, J = 6.3 Hz, OCHCH_3, 3H), 4.02-4.07 (m, OCHCH$_3$, 1H), 4.22-4.27 (m, CH_2OCO, 2H), 6.11 (s, NCH_2O, 2H), 7.33-7.36 (m, *H*-3, *H*-5 phenyl, *H* benzotriazole overlapping, 3H), 7.41-7.44 (t, J = 8.0 Hz, *H*-4 phenyl, 1H), 7.50-7.53 (t, J = 7.4 Hz, *H* benzotriazole, 1H), 7.65-7.67 (d, J = 8.3 Hz, *H* benzotriazole, 1H), 7.75-7.77 (d, with fine structure, *H*-2, *H*-6 phenyl, 2H), 8.03-8.05 (d, J = 8.3 Hz, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 16.38 (CH$\underline{C}$H$_3$), 67.18 ($\underline{C}$H$_2$OCO), 72.11 ($\underline{C}$HCH$_3$), 75.37 (NCH$_2$), 109.91 (C

benzotriazole), 119.94 (C benzotriazole), 124.33 (C benzotriazole), 127.90 (C benzotriazole), 128.26 (phenyl C), 129.44 (phenyl C), 129.56 (phenyl C), 132.62 (C benzotriazole), 132.89 (phenyl C), 146.40 (C benzotriazole), 166.07 (C=O). MS (EI) *m/z* (%) 311 M^+ (0.5), 195 (3), 180 (3), 163 (26), 132 (14), 105 (100), 77 (47), 51 (11), 18 (6).

1-[(1-Hydroxyprop-2-yloxy)methyl]benzotriazole (**2.68**) from 76% pure 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67**

An aqueous solution of ammonia (25%, 30 ml) was added to a solution of 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67** (378 mg, 1.2 mmol) in methanol (30 ml). The reaction mixture was stirred overnight at room temperature, after which it was concentrated at 50°C under reduced pressure to a volume of approximately 20 ml. The mixture was extracted twice both with toluene (2 × 20 ml) and $CHCl_3$ (2 × 20 ml). Each extract was dried separately over anhydrous $MgSO_4$, the drying agent was filtered off, and the filtrates concentrated to dryness under reduced pressure at 50°C. Crude 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68** was obtained from both the toluene (64 mg, 25%) and the chloroform (198 mg, 78%) extracts, both in an isomeric purity of 80% (HPLC, Method 6). [Characterization was only done on the purer 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68**, the synthesis of which is described below].

1-[(1-Hydroxyprop-2-yloxy)methyl]benzotriazole (**2.68**) from 99% pure 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67**

Concentrated ammonia solution (25%, 28 ml) was added to a solution of 99% pure 1-[(1-benzoyloxyprop-2-yloxy)methyl]benzotriazole **2.67** (353 mg, 1.1 mmol) in methanol (28 ml) and the mixture was stirred overnight at room temperature, after which the reaction mixture was concentrated under reduced pressure at 50°C to a volume of approximately 30 ml. The mixture was extracted twice with diethyl ether (2 × 30 ml), and once with $CHCl_3$ (30 ml). All the extracts were combined, dried over anhydrous $MgSO_4$, the drying agent was filtered off, and the filtrate concentrated to dryness to give 264 mg of crude material. This material was chromatographed over silica gel (8 g) using initially petroleum ether/ethyl acetate (2:1) as the

mobile phase. The first fraction (35 ml) was discarded, and elution was continued with petroleum ether/ethyl acetate (1:1). The second fraction (40 ml) was discarded, and the product was collected in the subsequent fraction (30 ml). This fraction was evaporated to dryness under reduced pressure at 80-90°C to give 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68** (214 mg, 91%) in a purity of 96% (HPLC, Method 6). ^{1}H NMR (600 MHz) δ: 1.06 (d, J = 6.3 Hz, OCHCH_3, 3H), 2.04 (bs, OH, 1H), 3.46-3.49 (dd, J_{gem} = 11.9 Hz, J_{vic} = 6.7 Hz, CH_2OH), 1H), 3.52-3.55 (dd, J_{gem} = 11.9 Hz, J_{vic} = 3.4 Hz, CH_2OH , 1H), 3.76-3.81 (m, OCHCH$_3$, 1H), 6.10, 6.13 (AB spin system, $^2J_{gem}$ = 11.5 Hz, NCH_2, 2H), 7.40-7.43 (t, with fine structure, H benzotriazole, 1H), 7.53-7.55 (t, with fine structure, H benzotriazole, 1H), 7.69-7.71 (d, J = 8.3 Hz, H benzotriazole, 1H), 8.07-8.09 (d, J = 8.3 Hz, H benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 15.86 (CH$\underline{C}$H$_3$), 66.13 ($\underline{C}$H$_2$OH), 75.41 (NCH$_2$), 75.57 ($\underline{C}$HCH$_3$), 109.72 (C benzotriazole), 120.06 (C benzotriazole), 124.42 (C benzotriazole), 128.03 (C benzotriazole), 132.70 (C benzotriazole), 146.32 (C benzotriazole). MS (EI) *m/z* (%) 207 M$^+$ (3), 177 (39), 133 (66), 120 (27), 104 (37), 91 (46), 77 (100), 59 (28), 51 (21), 31 (24), 29 (4).

*1-[(1-*p*-Toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole* (**2.69**) from 80% pure 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68**

Triethylamine (0.1 ml) and *p*-toluenesulphonyl chloride (126 mg, 0.7 mmol) were added to a solution of the crude $CHCl_3$ extract of 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68** (130 mg, approximately 0.6 mmol) in dry dichloromethane (6 ml). The mixture was refluxed and stirred on a hot plate for 6 hours, after which TLC (Methods 6 or 7) showed the presence of the unreacted starting compound **2.68**. Additional *p*-TsCl (20 mg, 0.1 mmol) was added to the reaction mixture and the mixture was further refluxed for several hours, after which unreacted **2.68** was still present. The reaction mixture was evaporated to dryness to give a crude product that was chromatographed over silica gel (8 g), using petroleum ether/ethyl acetate (10:1, 5:1, 3:1), and finally $CHCl_3$ as mobile phases. Only the fraction eluted with $CHCl_3$ contained 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69** (83 mg, 36%) in an isomeric purity of 98% [HPLC Method 2(b)]. ^{1}H NMR (300 MHz) δ: 0.94 (d, J = 6.2 Hz, OCHCH_3, 3H), 2.39 (s, Ts-CH_3, 3H), 3.79-3.88 (m, OCHCH$_3$ and CH_2OTs overlap,

3H), 5.96 (s, NCH_2O, 2H), 7.26-7.29, 7.65-7.68 (m, AA'BB' spin system, Ts aromatic *H*, 4H), 7.36-7.41 (ddd, $^3J_{vic}$ = 8.3 Hz, *H* benzotriazole, 1H), 7.47-7.53 (ddd, $^3J_{vic}$ = 8.3 Hz, *H* benzotriazole, 1H), 7.60-7.64 (d, with fine structure, *H* benzotriazole, 1H), 8.03-8.07 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (75 MHz) δ: 16.05 (CH$\underline{C}H_3$), 21.45 (Ts-$\underline{C}H_3$), 71.92 ($\underline{C}H_2$OTs), 71.98 ($\underline{C}$HCH$_3$), 75.65 (NCH$_2$), 110.01 (C benzotriazole), 120.16 (C benzotriazole), 124.60 (C benzotriazole), 127.99 (Ts aromatic C), 128.24 (C benzotriazole), 130.02 (Ts aromatic C), 132.83 (Ts aromatic C), 132.93 (C benzotriazole), 145.17 (Ts aromatic C), 146.61 (C benzotriazole). HRMS: *m/z* M^+ = 361.1117, calc. for $C_{17}H_{19}O_4N_3S$ = 361.1096. MS (EI) *m/z* (%) 361 M^+ (59), 242 (16), 206 (45), 189 (14), 155 (98), 132 (81), 91 (100), 65 (12).

*1-[(1-*p*-Toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole* (**2.69**) from 96% pure 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68**

A mixture of 1-[(1-hydroxyprop-2-yloxy)methyl]benzotriazole **2.68** (100 mg, 0.5 mmol), *p*-toluenesulphonyl chloride (126 mg, 0.7 mmol) and triethylamine (0.1 ml) in dry dichloromethane (5 ml) was refluxed and stirred in an oil bath at 120°C for 6 hours. Additional *p*-TsCl (68 mg, 0.3 mmol) was added to the reaction mixture and reflux was continued for a further 3 hours. The reaction mixture was evaporated to dryness under reduced pressure at 50°C and chromatographed over silica gel (8 g), using initially petroleum ether/ethyl acetate (5:1) as mobile phase. The first fraction (55 ml) was discarded, and elution was continued with petroleum ether/ethyl acetate (1:1). The second fraction (15 ml) was discarded, and the product was collected in the subsequent fraction (15 ml). This fraction was evaporated to dryness under reduced pressure at 80-90°C to give 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69** (153 mg, 91%) in a 98% purity [HPLC Method 2(b)]. ^{1}H NMR (600 MHz) δ: 1.0 (d, *J* = 6.1 Hz, OCHCH_3, 3H), 2.45 (s, Ts-CH_3, 3H), 3.86-3.89 (m, OC*H*CH$_3$, 1H), 3.91-3.94 (m, CH_2OTs, 2H), 6.0 (s, NCH_2O, 2H), 7.30-7.32, 7.68-7.70 (m, AA'BB' spin system, Ts aromatic *H*, 4H), 7.40-7.43 (t, with fine structure, *H* benzotriazole, 1H), 7.52-7.54 (t, with fine structure, *H* benzotriazole, 1H), 7.64-7.65 (d, *J* = 8.3 Hz, *H* benzotriazole, 1H), 8.07-8.08 (d, *J* = 8.3 Hz, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ:

16.25 ($CH\underline{C}H_3$), 21.63 (Ts-$\underline{C}H_3$), 71.93 ($\underline{C}H_2OTs$), 71.98 ($\underline{C}HCH_3$), 75.64 (NCH_2), 109.88 (C benzotriazole), 120.0 (C benzotriazole), 124.42 (C benzotriazole), 127.81 (Ts aromatic C), 128.06 (C benzotriazole), 129.84 (Ts aromatic C), 132.63 (Ts aromatic C), 132.70 (C benzotriazole), 144.93 (Ts aromatic C), 146.37 (C benzotriazole). MS (EI) *m/z* (%) 361 M^+ (33), 242 (8), 206 (19), 173 (6), 155 (92), 132 (42), 120 (30), 104 (24), 91 (100), 77 (43), 65 (16), 39 (5).

1-[(1-Iodoprop-2-yloxy)methyl]benzotriazole (**2.70**)

A mixture of 98% pure 1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]benzotriazole **2.69** (48 mg, 0.13 mmol) and sodium iodide (23 mg, 0.15 mmol) in acetone (1 ml) was heated and stirred in a sealed vial (5 ml) in an oil bath at 100-130°C for 1.5 hours. After evaporation of the solvent with a stream of nitrogen, the reaction product was chromatographed over silica gel (5 g) with petroleum ether/ethyl acetate (3:1) to give 1-[(1-iodoprop-2-yloxy)methyl]benzotriazole **2.70** as an oil (36 mg, 86%) in a 99% purity [HPLC Method 2(b)]. ^{1}H NMR (600 MHz) δ: 1.2 (d, J = 6.1 Hz, OCHCH_3, 3H), 3.11-3.14 (dd, J_{gem} = 10.6 Hz, J_{vic} = 5.5 Hz, C*H*HI, 1H), 3.17-3.20 (dd, J_{gem} = 10.6 Hz, J_{vic} = 4.8 Hz, CH*H*I, 1H), 3.59-3.63 (m, OC*H*CH_3, 1H), 6.07 (s, NCH_2, 2H), 7.41-7.44 (t, with fine structure, *H* benzotriazole), 1H), 7.54-7.57 (t, with fine structure, *H* benzotriazole, 1H), 7.72-7.74 (d, J = 8.3 Hz, *H* benzotriazole, 1H), 8.08-8.10 (d, J = 8.3 Hz, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 10.23 (CH_2I), 20.29 ($CH\underline{C}H_3$), 73.20 (NCH_2), 75.15 ($\underline{C}HCH_3$), 110.07 (C benzotriazole), 120.02 (C benzotriazole), 124.45 (C benzotriazole), 128.02 (C benzotriazole), 132.74 (C benzotriazole), 146.41 (C benzotriazole). MS (EI) *m/z* (%) 317 M^+ (45), 289 (10), 259 (22), 231 (31), 190 (61), 169 (100), 132 (52), 119 (47), 77 (67), 41 (65), 27 (5).

1-(2-Hydroxyprop-1-yl)benzotriazole (**2.71**)

A mixture of benzotriazole (200 mg, 1.7 mmol), anhydrous K_2CO_3 (720 mg, 5.2 mmol) and 1-bromo-2-propanol (99.7% isomeric purity) (413 mg, 2.5 mmol) in acetonitrile (20 ml) was refluxed and stirred on a hot plate for 5.5 hours. Additional 1-bromo-2-propanol (383 mg, 2.3 mmol) was added to the reaction mixture, and the mixture was refluxed for another 5.5 hours.

The reaction mixture was filtered and the filtrate evaporated to dryness at 85°C under reduced pressure. The crude product was chromatographed over silica gel (10 g), using initially petroleum ether/ethyl acetate (3:1) as the mobile phase. The first fraction (33 ml) was discarded, and elution was continued with petroleum ether/ethyl acetate (1:1). The second fraction (25 ml) was discarded, and elution was continued with ethyl acetate. The first 20 ml (fraction 3) contained impurities, while the subsequent fractions 4 and 5 (5 ml each) contained the product (most intense spot on TLC). Fraction 4 was re-chromatographed on silica gel (5 g), using petroleum ether/ethyl acetate (1:1) as the mobile phase. The first 30 ml was discarded and the subsequent product-containing fraction (30 ml) was combined with fraction 5 obtained from the first column purification procedure. Evaporation to dryness gave 1-(2-hydroxyprop-1-yl)benzotriazole **2.71** as an oily substance which later solidified into a solid wax (141 mg, 47%). ^{1}H NMR (600 MHz) δ: 1.34-1.35 (d, J = 6.1 Hz, CHC*H*$_3$, 3H), 3.38 (s, O*H*, 1H), 4.45-4.53 (C*H*-OH, NC*H*H overlap, 2H), 4.62-4.64 (AB spin system, dd, J_{gem} = 13.5 Hz, J_{vic} = 2.8 Hz, NCH*H*, 1H), 7.27-7.30 (t, with fine structure, *H* benzotriazole, 1H), 7.44-7.47 (t, with fine structure, *H* benzotriazole, 1H), 7.59-7.61 (d, with fine structure, *H* benzotriazole, 1H), 7.84-7.85 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 20.62 (CH$\underline{C}$H$_3$), 55.16 (NCH$_2$), 66.97 ($\underline{C}$HCH$_3$), 109.93 (C benzotriazole), 119.45 (C benzotriazole), 123.96 (C benzotriazole), 127.34 (C benzotriazole), 133.69 (C benzotriazole), 145.35 (C benzotriazole). MS (EI) *m/z* (%) 177 M$^+$ (35), 132 (41), 104 (77), 91 (25), 77 (100), 51 (26), 45 (37), 18 (12). A small amount of the less polar product 2-(2-hydroxyprop-1-yl)benzotriazole **2.72** was also isolated (85 mg). ^{13}C NMR δ: 19.91 (CH$\underline{C}$H$_3$), 62.78 (NCH$_2$), 66.62 ($\underline{C}$HCH$_3$), 118.02 (C benzotriazole), 126.73 (C benzotriazole), 144.35 (C benzotriazole).

*1-(2-*p*-Toluenesulphonyloxyprop-1-yl)benzotriazole* (**2.73**)

A mixture of 1-(2-hydroxyprop-1-yl)benzotriazole **2.71** (85 mg, 0.48 mmol), triethylamine (0.1 ml) and *p*-toluenesulphonyl chloride (123 mg, 0.65 mmol) in dry dichloromethane (2 ml) was refluxed and stirred in an oil bath at 140°C for 4 hours. The reaction mixture was reduced in volume with a stream of nitrogen to approximately 1 ml, additional triethylamine (0.1 ml) was added to the mixture and heating was continued at 150°-160°C for 2 hours. Additional *p*-

toluenesulphonyl chloride (50 mg) was added to the reaction mixture and heating was continued for another 1 hour at 140°C. The reaction mixture was chromatographed over silica gel (8 g), using $CHCl_3$, followed by a gradient of petroleum ether/ethyl acetate (3:1, 2:1 and 1:1) as mobile phases. Fractions containing a product having the same R_f value as the main component in the crude reaction mixture were combined and evaporated to dryness to give a white crystalline solid (59 mg, 37%). Crystallisation from ethyl acetate gave 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73** (41 mg, 26%) in a purity of 97% [HPLC Method 2(b)], m.p. 151.5-152°C, ^{1}H NMR (600 MHz) δ: 1.49 (d, J = 6.3 Hz, CHC*H*$_3$, 3H), 2.34 (s, Ts-C*H*$_3$, 3H), 4.67-4.71 (AB spin system, dd, J_{gem} = 15.0 Hz, J_{vic} = 7.5 Hz, NC*H*H, 1H), 4.74-4.77 (AB spin system, dd, J_{gem} = 15.0 Hz, J_{vic} = 3.7 Hz, NCH*H*, 1H), 4.98-5.03 (m, C*H*OTs, 1H), 6.98-7.0, 7.31-7.32 (m, AA'BB' spin system, Ts aromatic *H*, 4H), 7.33-7.36 (t, with fine structure, *H* benzotriazole, 1H), 7.44-7.47 (t, with fine structure, *H* benzotriazole, 1H), 7.50-7.52 (d, with fine structure, *H* benzotriazole, 1H), 7.93-7.95 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 18.94 (CH<u>C</u>H$_3$), 21.58 (Ts-<u>C</u>H$_3$), 52.21 (NCH$_2$), 77.50 (<u>C</u>HOTs), 109.69 (C benzotriazole), 119.76 (C benzotriazole), 123.86 (C benzotriazole), 126.97 (Ts aromatic C), 127.69 (C benzotriazole), 129.52 (Ts aromatic C), 132.37 (Ts aromatic C), 133.31 (C benzotriazole), 144.69 (Ts aromatic C), 145.62 (C benzotriazole). HRMS: *m/z* M^+ = 331.0978, calc. for $C_{16}H_{17}O_3N_3S$ = 331.0991. MS (EI) *m/z* (%) 331 M^+ (38), 223 (13), 194 (15), 155 (100), 132 (56), 104 (13), 91 (45) 65 (6).

1-(2-Iodoprop-1-yl)benzotriazole (**2.74**)

A mixture of 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73** (25 mg, 0.075 mmol) and sodium iodide (17 mg, 0.113 mmol) in acetone (0.8 ml) was heated with stirring in a sealed vial in an oil bath at 120-125°C for 2.5 hours. After evaporation of the solvent, the reaction mixture was chromatographed over silica gel (8 g) using petroleum ether/ethyl acetate (3:1) as mobile phase, to give 1-(2-iodoprop-1-yl)benzotriazole **2.74** as a off-white semi-solid (17.5 mg, 81%) in a purity of 99% [HPLC Method 2(b)]. ^{1}H NMR (300 MHz) δ: 1.89-1.92 (d, J = 6.9 Hz, CHC*H*$_3$, 3H), 4.61-4.73 (m, C*H*I, 1H), 4.91 (AB spin system, dd, J_{gem} = 14.6 Hz, J_{vic} = 8.2 Hz, NC*H*H, 1H), 5.06 (AB spin system, dd, J_{gem} = 14.6 Hz, J_{vic} = 6.6 Hz, NCH*H*, 1H),

7.41-7.47 (t, with fine structure, *H* benzotriazole, 1H), 7.54-7.60 (t, with fine structure, *H* benzotriazole, 1H), 7.61-7.64 (d, with fine structure, *H* benzotriazole, 1H), 8.12-8.15 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (75 MHz) δ: 20.10 (I-$\underline{C}HCH_3$), 25.55($CH\underline{C}H_3$), 57.20 (NCH_2), 109.35 (C benzotriazole), 120.35 (C benzotriazole), 124.26 (C benzotriazole), 127.88 (C benzotriazole), 133.22 (C benzotriazole), 145.93 (C benzotriazole). MS (EI) *m/z* (%) 287 M^+ (100), 258 (10), 230 (78), 203 (20), 160 (18), 132 (63), 104 (18), 77 (44), 41 (37), 27 (6).

1-(3-Hydroxy-2-methylprop-1-yl)benzotriazole (**2.76**)

A mixture of benzotriazole (100 mg, 0.84 mmol), anhydrous K_2CO_3 (360 mg) and (*S*)-(+)-3-bromo-2-methyl-1-propanol (97%, 192 mg, 1.25 mmol) in acetonitrile (10 ml) was refluxed and stirred on a hot plate for 1 hour. The reaction mixture was filtered and the filtrate evaporated to dryness. Column chromatography over silica gel (5 g), using petroleum ether/ethyl acetate (1:1) as mobile phase, gave two fractions, of which the more polar fraction (lower R_f on TLC) yielded 1-(3-hydroxy-2-methylprop-1-yl)benzotriazole **2.76** (70 mg, 44%). ^{1}H NMR (300 MHz) δ: 1.01 (d, J = 7.0 Hz, CHC*H*$_3$, 3H), 2.38-2.49 (m, CH_2C*H*, O*H* overlapping, 2H), 3.47-3.63 (m, C*H*$_2$OH, 2H), 4.60-4.67 (AB spin system, dd, J_{gem} = 14.2 Hz, J_{vic} = 6.5 Hz, NC*H*H, 1H), 4.75-4.82 (AB spin system, dd, J_{gem} = 14.2 Hz, J_{vic} = 6.7 Hz, NCH*H*, 1H), 7.38-7.44 (t, with fine structure, *H* benzotriazole, 1H), 7.50-7.56 (t, with fine structure, *H* benzotriazole, 1H), 7.63-7.66 (d, with fine structure, *H* benzotriazole, 1H), 8.08-8.12 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (75 MHz) δ: 14.45 ($CH\underline{C}H_3$), 36.43 ($\underline{C}HCH_3$), 50.09 (NCH_2), 64.29 (CH_2OH), 109.65 (C benzotriazole), 120.02 (C benzotriazole), 124.06 (C benzotriazole), 127.52 (C benzotriazole), 133.72 (C benzotriazole), 145.91 (C benzotriazole). MS (EI) *m/z* (%) 191 M^+ (51), 161 (8), 132 (51), 119 (19), 91 (100), 77 (95), 55 (25), 31 (38), 27 (13).

*1-(2-Methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole* (**2.77**)

This compound was prepared from 1-(3-hydroxy-2-methylprop-1-yl)benzotriazole **2.76** (55 mg, 0.29 mmol) and *p*-toluenesulphonyl chloride (85 mg, 0.45 mmol) according to the

procedure used for the preparation of 1-(2-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.73**. Column chromatography over silica gel (7 g) and elution with $CHCl_3$, followed by petroleum ether/ethyl acetate (1:1), gave an impure product. A second purification over silica gel (6 g), using petroleum ether/ethyl acetate (2:1) as mobile phase, gave pure 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.77** (49 mg, 50%). ^{1}H NMR (300 MHz) δ: 1.02 (d, J = 6.9 Hz, CHC*H*$_3$, 3H), 2.45 (s, Ts-C*H*$_3$, 3H), 2.63-2.73 (m, CH_2C*H*, 1H), 3.95 (d, J = 5.4 Hz, C*H*$_2$OTs, 2H), 4.49-4.56 (AB spin system, dd, J_{gem} = 14.2 Hz, J_{vic} = 7.0 Hz, NC*H*H, 1H), 4.63-4.71 (AB spin system, dd, J_{gem} = 14.2 Hz, J_{vic} = 6.7 Hz, NCH*H*, 1H), 7.33-7.36, 7.76-7.78 (m, AA'BB' spin system, Ts aromatic *H*, 4H), 7.39-7.45 (m, *H* benzotriazole, 1H), 7.53-7.55 (m, *H* benzotriazole, 2H), 8.08-8.10 (d, with fine structure, *H* benzotriazole, 1H). ^{13}C NMR (75 MHz) δ: 14.28 (CH$\underline{C}$H$_3$), 21.44 (Ts-$\underline{C}$H$_3$), 34.01 ($\underline{C}$HCH$_3$), 49.57 (NCH$_2$), 71.44 ($\underline{C}$H$_2$OTs), 109.32 (C benzotriazole), 120.15 (C benzotriazole), 124.11 (C benzotriazole), 127.72 (C benzotriazole), 128.01 (Ts aromatic C), 130.09 (Ts aromatic C), 132.61 (Ts aromatic C), 133.50 (C benzotriazole), 145.29 (Ts aromatic C), 145.95 (C benzotriazole). MS (EI) *m/z* (%) 345 M^+ (12), 221 (6), 190 (14), 172 (13), 144 (23), 120 (27), 91 (100), 77 (63), 65 (26), 18 (32).

1-(3-Iodo-2-methylprop-1-yl)benzotriazole (**2.78**)

This compound was prepared from 1-(2-methyl-3-*p*-toluenesulphonyloxyprop-1-yl)benzotriazole **2.77** (17 mg, 0.05 mmol) and sodium iodide (10 mg, 0.07 mmol) according to the procedure used for the preparation of 1-(2-iodoprop-1-yl)benzotriazole **2.74**. Column chromatography over silica gel gave 1-(3-iodo-2-methylprop-1-yl)benzotriazole **2.78** (12 mg, 82%). ^{1}H NMR (600 MHz) δ: 1.09 (d, J = 6.8 Hz, CHC*H*$_3$, 3H), 2.31-2.36 (m, CH_2C*H*, 1H), 3.15 (AB spin system, dd, J_{gem} = 10.3 Hz, J_{vic} = 4.9 Hz, C*H*HI, 1H), 3.23 (AB spin system, dd, J_{gem} = 10.3 Hz, J_{vic} = 5.4 Hz, CH*H*I, 1H), 4.51 (AB spin system, dd, J_{gem} = 14.0 Hz, J_{vic} = 6.7 Hz, NC*H*H, 1H), 4.65 (AB spin system, dd, J_{gem} = 14.2 Hz, J_{vic} = 7.1 Hz, NCH*H*, 1H), 7.37-7.40 (t, J = 7.5 Hz, *H* benzotriazole, 1H), 7.50-7.53 (t, J = 7.6 Hz, *H* benzotriazole, 1H), 7.62 (d, J = 8.3 Hz, *H* benzotriazole, 1H), 8.07 (d, J = 8.3 Hz, *H* benzotriazole, 1H). ^{13}C NMR (150 MHz) δ: 12.26 (CH$\underline{C}$H$_3$), 18.91 (CH_2I), 35.63 ($\underline{C}$HCH$_3$), 53.00 (NCH$_2$), 109.39 (C

benzotriazole), 120.12 (C benzotriazole), 123.99 (C benzotriazole), 127.50 (C benzotriazole), 133.35 (C benzotriazole), 145.82 (C benzotriazole). MS (EI) *m/z* (%) 301 M^+ (34), 231 (48), 203 (5), 174 (54), 132 (81), 104 (38), 77 (100), 41 (36), 18 (36).

6.3.3.2 Synthesis of the alkylating agents and the 2-methyl-4-nitroimidazole derivatives

1-[(1-Benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.80**)

A mixture of 2-methyl-5-nitroimidazole **2.79** (350 mg, 2.7 mmol), anhydrous K_2CO_3 (1 g) and acetonitrile (70 ml) was stirred at room temperature for 30 minutes. A solution of freshly prepared isomerically impure 2-chloromethoxyprop-1-yl benzoate **2.56** (783 mg, 3.4 mmol) in acetonitrile (9 ml) was added to the resultant yellow suspension. The reaction mixture was stirred overnight at room temperature, after which it was filtered and the filtrate evaporated to dryness. The filtrate residue was chromatographed over silica gel (26 g), using ethyl acetate as mobile phase. The major, least polar product was isolated to give an off-white solid (768 mg). It was crystallised from methanol to give 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80** as white crystals (289 mg, 33%) in a purity of 98% [HPLC Method 5], m.p. 139-139.5°C, ^{1}H NMR (300 MHz) δ: 1.27-1.30 (d, J = 6.4 Hz, OCHCH_3, 3H), 2.47 (s, CH_3 imidazole, 3H), 3.93-4.02 (m, OCHCH$_3$, 1H), 4.27-4.40 (m, CH_2OCO, 2H), 5.40, 5.43 (AB spin system, $^2J_{gem}$ = 11.0 Hz, NCH_2, 2H), 7.48-7.53 [t (with fine structure), *H*-3, *H*-5 phenyl, 2H], 7.61-7.66 [t (with fine structure), *H*-4 phenyl, 1H], 7.83 (s, *H* imidazole, 1H), 7.99-8.02 [d (with fine structure), *H*-2, *H*-6 phenyl, 2H]. ^{13}C NMR (75 MHz) δ: 12.83 (imidazole CH_3), 16.66 (CHCH_3), 67.04 (CH_2OCO), 72.98 (CHCH$_3$), 74.88 (NCH$_2$), 119.56 (C-5 imidazole), 128.73 (phenyl C), 129.50 (phenyl C), 129.63 (phenyl C), 133.65 (phenyl C), 145.46 (C imidazole), 147.08 (C imidazole), 166.48 (C=O). MS (EI) *m/z* (%) 319 M^+ (1), 193 (7), 163 (15), 141 (7), 105 (100), 77 (22), 43 (8).

1-[(1-Hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.82**)

Concentrated ammonia solution (25%, 8 ml) was added to a solution of 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80** (200 mg, 0.62 mmol) in methanol (15 ml),

and the mixture was stirred overnight at room temperature after which the reaction mixture was evaporated to near dryness under reduced pressure at 60°C. Residual water was azeotropically distilled off with acetonitrile. The residue was triturated with $CHCl_3$ (20 ml), the residue was filtered off and the filtrate evaporated to dryness. The brownish filtrate residue was chromatographed over silica gel (4 g), using ethyl acetate as mobile phase, to give pure 1-[(1-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.82** (126 mg, 93%). ^{1}H NMR (300 MHz) δ: 1.12-1.14 (d, J = 6.4 Hz, OCHC*H*$_3$, 3H), 2.51 (s, C*H*$_3$ imidazole, 3H), 3.58-3.67 (m, C*H*$_2$OH, 2H), 3.70-3.79 (m, OC*H*CH$_3$, 1H), 5.44, 5.53 (AB spin system, $^2J_{gem}$ = 10.8 Hz, NC*H*$_2$, 2H), 7.89 (s, *H* imidazole, 1H). ^{13}C NMR (75 MHz) δ: 12.82 (imidazole C̲H$_3$), 16.12 (CHC̲H$_3$), 66.68 (CH$_2$OH), 75.28 (NCH$_2$), 75.78 (C̲HCH$_3$), 119.98 (C-5 imidazole), 145.62 (C imidazole), 146.57 (C imidazole). MS (EI) *m/z* (%) 215 M$^+$ (4), 185 (3), 156 (2), 141 (11), 127 (18), 98 (4), 81 (13), 59 (100), 43 (53), 28 (4).

*2-Methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole* (**2.83**)

This compound was prepared from 1-[(1-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.82** (97 mg, 0.45 mmol) and *p*-toluenesulphonyl chloride (112 mg, 0.58 mmol) according to the procedure used for the preparation of 1-[(1-*p*-toluenesulphonyl-oxyprop-2-yloxy)methyl]benzotriazole **2.69**. The reaction mixture was refluxed in an oil bath at 100°C for 4.5 hours, and column chromatography was carried out over silica gel (6 g), using initially petroleum ether/ethyl acetate (1:1) as the mobile phase. The first fraction (13 ml) was discarded, and elution was continued with petroleum ether/ethyl acetate (1:3). The second fraction (15 ml) was discarded, and the product was collected in the subsequent fraction (24 ml). 2-Methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.83** was obtained as a white solid (152 mg, 91%), m.p. 97.5-98°C, ^{1}H NMR (600 MHz) δ: 1.12 (d, J = 6.4 Hz, OCHC*H*$_3$, 3H), 2.46 (2s, C*H*$_3$ imidazole and Ts-C*H*$_3$ overlap, 6H), 3.82-3.87 (m, OC*H*CH$_3$, 1H), 3.95 (AB spin system, dd, J_{gem} = 10.9 Hz, J_{vic} = 7.5 Hz, C*H*HOTs, 1H), 4.01 (AB spin system, dd, J_{gem} = 10.9 Hz, J_{vic} = 3.2 Hz, CH*H*OTs, 1H), 5.33, 5.35 (AB spin system, $^2J_{gem}$ = 11.0 Hz, NC*H*$_2$, 2H), 7.37, 7.76 (m, AA'BB' spin system, Ts aromatic *H*, 4H), 7.73 (s, *H* imidazole, 1H). ^{13}C NMR (150 MHz) δ: 13.02 (imidazole C̲H$_3$), 16.64 (CHC̲H$_3$), 21.63 (Ts-

$\underline{C}H_3$), 72.39 ($\underline{C}H_2OTs$), 72.72 ($\underline{C}HCH_3$), 75.32 (NCH_2), 119.48 (C-5 imidazole), 127.77 (Ts aromatic C), 130.02 (Ts aromatic C), 132.41 (Ts aromatic C), 145.32 (C imidazole), 145.45 (Ts aromatic C), 146.56 (C imidazole). HRMS: *m/z* M^+ = 369.0997, calc. for $C_{15}H_{19}O_6N_3S$ = 369.0994. MS (EI) *m/z* (%) 369 M^+ (3), 243 (16), 213 (60), 198 (5), 155 (100), 141 (10), 91 (23).

2-Methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole (**2.85**)

Neat trifluoromethanesulphonic anhydride (14 µl, 0.08 mmol) was added to a chilled (-10°C) solution of 1-[(1-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.82** (15 mg, 0.07 mmol) in dry acetonitrile (0.3 ml). The reaction mixture was refrigerated at -10°C for approximately 1 hour, after which the solvent was evaporated with a stream of nitrogen. Ice water (1 ml) and dichloromethane (1 ml) was added to the residue, the aqueous phase was made basic with sodium carbonate solution, the mixture stirred well and the layers separated. The aqueous phase was extracted three times with dichloromethane (0.5 ml) and the combined dichloromethane extracts were dried over anhydrous $MgSO_4$. The drying agent was filtered off and the filtrate concentrated with a stream of nitrogen to give 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85** as a white solid (25 mg, 100% yield). This solid was immediately dissolved in dry dichloromethane (1.25 ml) and refrigerated at -10°C.

1-[(1-Iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.87**)

A mixture of 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.83** (25 mg, 0.07 mmol) and sodium iodide (12 mg, 0.08 mol) in acetone (1.5 ml) was heated under reflux in an oil bath at 120-140°C for 1.5 hours. Additional sodium iodide (5.8 mg) in acetone (0.3 ml) was added to the reaction mixture and heating was continued for a further 45 minutes. The reaction mixture was chromatographed over silica gel (5 g), using ethyl acetate/hexane/chloroform (70:15:15) as mobile phase, to give pure 1-[(1-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87** (18 mg, 82%). ^{1}H NMR (600 MHz) δ: 1.27 (d, *J* = 6.2 Hz, OCHC*H*$_3$, 3H), 2.54 (s, C*H*$_3$ imidazole, 3H), 3.15-3.21 (m, C*H*$_2$I, 2H), 3.59-3.64 (m,

OC*H*CH_3, 1H), 5.34, 5.37 (AB spin system, $^2J_{gem}$ = 11.0 Hz, NCH_2, 2H), 7.80 (s, *H* imidazole, 1H). ^{13}C NMR (150 MHz) δ: 9.03 (CH_2I), 13.35 (imidazole $\underline{C}H_3$), 20.43 (CH$\underline{C}H_3$), 74.58 (NCH_2), 74.70 ($\underline{C}HCH_3$), 119.52 (C-5 imidazole), 145.52 (C imidazole), 146.63 (C imidazole). MS (EI) *m/z* (%) 325 M^+ (3), 295 (1), 199 (19), 169 (100), 141 (18), 127 (1), 98 (2), 41 (20), 39 (3).

2-Bromo-1-propanol (**2.53**)

A solution of lithium aluminium hydride (LAH) in tetrahydrofuran (1M, 70 ml, 70 mmol) was placed in a thoroughly dried 250 ml Erlenmeyer flask and cooled to -10°C. Concentrated sulphuric acid (1.9 ml, 35 mmol) was added dropwise with stirring to the LAH at such a rate that the temperature of the mixture did not exceed 4°C. After the addition was completed, the mixture was cooled to -5°C while being stirred and treated with a solution of 2-bromopropionic acid (4.5 g, 29.4 mmol) in anhydrous tetrahydrofuran (30 ml) at such a rate that the temperature of the mixture did not exceed 12°C. After the addition was completed, the temperature was allowed to increase to room temperature and stirring was continued for 2.5 hours. The mixture was cooled to 5°C, and water/THF (1:1, 10 ml) was added dropwise to the mixture while maintaining the temperature of the mixture at 0-5°C. Stirring was continued for 10-15 minutes. Diethyl ether (100ml) and sodium hydroxide solution (15%, 13 ml) were added to the reaction mixture, and stirring was continued for another 10 minutes. The mixture was filtered and the filtrate concentrated at 45-50°C under reduced pressure to a volume of approximately 2 ml. Dichloromethane (20 ml) and anhydrous $MgSO_4$ were added, the mixture was stirred for 25 minutes, filtered, and the filtrate concentrated at 30-35°C under reduced pressure to give a colourless liquid. Residual solvent was removed by drying the liquid under vacuum at 55°C for a few hours, giving 2-bromo-1-propanol **2.53** (0.86 g, 21%) in an isomeric purity of 95% (GC Method 1). The product and its isomeric impurity were identified by comparing the retention times on the GC chromatogram with those obtained from a commercially available mixture of the isomers 1-bromo-2-propanol **2.52** and 2-bromo-1-propanol **2.53**.

1-Hydroxyprop-2-yl benzoate (**2.55**) from 95% isomerically pure 2-bromo-1-propanol **2.53**

A mixture of sodium benzoate (0.3 g, 2.08 mmol) and 2-bromo-1-propanol **2.53** (460 mg, 3.3 mmol) in dimethylformamide (5 ml) was heated and stirred in a sealed vial (10 ml) in an oil bath at 80-100°C for an hour. Additional 2-bromo-1-propanol (240 mg) and sodium benzoate (0.3 g) were added to the reaction mixture which was heated at 100-117°C for a further 6 hours and then allowed to cool to room temperature. The solid matrerial was filtered off, and the filtrate evaporated to dryness under reduced pressure at 100°C. Further work-up and purification, carried out as described for the preparation of 2-hydroxyprop-1-yl benzoate **2.54**, gave 1-hydroxyprop-2-yl benzoate **2.55** (542 mg, 75%) in an isomeric purity of 66% [HPLC Method 3(a)]. ^{1}H NMR (300 MHz) δ: 1.37-1.39 (d, J = 6.5 Hz, CHC*H*$_3$, 3H), 2.1 (bs, O*H*, 1H), 3.75-3.86 (m, C*H*$_2$OH, 2H), 5.25-5.31 (m, CO$_2$-C*H*, 1H), 7.46-7.51 (t, with fine structure, *H*-3, *H*-5 phenyl, 2H), 7.58-7.64 (t, with fine structure, *H*-4 phenyl, 1H), 8.08-8.11 (d, with fine structure, H-2, H-6 phenyl, 2H). ^{13}C NMR (75 MHz) δ: 16.12 (CH<u>C</u>H$_3$), 66.05 (CH$_2$OH), 72.71 (<u>C</u>HCH$_3$), 128.52 (phenyl C), 129.79 (phenyl C), 130.41 [phenyl C (*ipso*)], 133.25 (phenyl C), 166.90 (C=O). MS (EI) *m/z* (%) 180 M$^+$ (0.2), 162 (2), 150 (3), 136 (5), 123 (11), 105 (100), 77 (37), 51 (13), 31 (5), 18 (4).

1-Chloromethoxyprop-2-yl benzoate (**2.57**) from **2.55** of 70% isomeric purity

This reagent was prepared from 1-hydroxyprop-2-yl benzoate **2.55** (595 mg, 3.3 mmol) and paraformaldehyde (113 mg, 3.76 mmol) according to the procedure used for the preparation of 2-chloromethoxyprop-1-yl benzoate **2.56**. The final product was recovered in dichloromethane (24 ml), with a solid content, calculated as described in the preparation of 2-chloromethoxyprop-1-yl benzoate **2.56,** of approximately 720 mg. The solution was refrigerated at -10°C.

1-[(2-Benzoyloxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.88**)

A mixture of 2-methyl-5-nitroimidazole **2.79** (255 mg, 2.0 mmol), anhydrous K$_2$CO$_3$ (765 mg) and acetonitrile (50 ml) was stirred at room temperature for 50 minutes. A solution of freshly prepared 1-chloromethoxyprop-2-yl benzoate **2.57** (approximately 720 mg, ~3.1 mmol) in

dichloromethane (24 ml) was added to the yellow suspension. The mixture was stirred for approximately 4.5 hours at room temperature, after which it was filtered and the filtrate evaporated to dryness. The crude reaction product was chromatographed over silica gel (8 g), using ethyl acetate as mobile phase. The fractions containing the target compound **2.88** were combined, evaporated to near dryness, and the residue dissolved in methanol (2.5 ml). On refrigerating this solution overnight at -10°C some crystals of the isomer 1-[(1-benzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.80** were formed. The mother liquor was carefully removed with a pasteur pipette, the crystals washed with a small portion of cold methanol, and the combined mother and wash liquors evaporated to dryness. The residue, highly enriched in **2.88**, was chromatographed over silica gel (20 g), using ethyl acetate/chloroform (1:1) as mobile phase. Pure fractions were combined to give 1-[(2-benzoyloxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.88** as an oil (200 mg, 31%), in 90% isomeric purity (HPLC Method 4). ^{1}H NMR (300 MHz) δ: 1.37 (d, J = 6.5 Hz, CHCH_3, 3H), 2.47 (s, CH_3 imidazole, 3H), 3.67 (d, J = 5.0 Hz, OCH_2CH, 2H), 5.32-5.42 (m, NCH_2 and CHCH$_3$ overlapping, 3H), 7.47-7.53 [t (with fine structure), H-3, H-5 phenyl, 2H], 7.60-7.66 [t (with fine structure), H-4 phenyl, 1H], 7.82 (s, H imidazole, 1H), 8.02-8.05 [d (with fine structure), H-2, H-6 phenyl, 2H]. ^{13}C NMR (75 MHz) δ: 12.82 (imidazole $\underline{C}$H$_3$), 16.35 (CH$\underline{C}$H$_3$), 68.91 ($\underline{C}$HCH$_3$), 71.51 (OCH$_2$), 76.49 (NCH$_2$), 119.64 (C-5 imidazole), 128.63 (phenyl C), 129.68 (phenyl C), 130.00 (phenyl C), 133.46 (phenyl C), 145.56 (C imidazole), 146.87 (C imidazole), 166.18 (C=O). MS (EI) *m/z* (%) 319 M$^+$ (1), 193 (10), 163 (17), 105 (100), 77 (12).

1-[(2-Hydroxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.89**)

This compound was prepared from 1-[(2-benzoyloxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.88** (184 mg, 0.57 mmol) according to the procedure used for the preparation of 1-[(1-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.82**, except for using double the molar excess of 25% ammonia that was required for the synthesis of **2.82**. The CHCl$_3$ extract was chromatographed over silica gel, using ethyl acetate, followed by ethyl acetate/methanol (20:1) as mobile phase. The target compound 1-[(2-hydroxyprop-1-

yloxy)methyl]-2-methyl-4-nitroimidazole **2.89** was obtained as a white crystalline substance (112 mg, 90%) in an isomeric purity of 89% (HPLC, Method 6). Recrystallisation from ethyl acetate (1.5 ml) at -10°C gave **2.89** as white crystals (68 mg) in 94% isomeric purity, m.p. 96.5-97.5°C, ^{1}H NMR (600 MHz) δ: 1.17 (d, J = 6.6 Hz, CHC*H*$_3$, 3H), 2.50 (s, C*H*$_3$ imidazole, 3H), 3.32-3.35 (dd, J_{gem} = 9.5 Hz, J_{vic} = 7.3 Hz, OC*H*HCH, 1H), 3.46-3.48 (dd, J_{gem} = 9.5 Hz, J_{vic} = 3.2 Hz, OCH*H*CH, 1H), 4.0 (m, C*H*OH, 1H), 5.36, 5.40 (AB spin system, $^2J_{gem}$ = 10.9 Hz, NC*H*$_2$, 2H), 7.81 (s, *H* imidazole, 1H). ^{13}C NMR (150 MHz) δ: 13.06 (imidazole C$\underline{H}_3$), 19.06 (CH$\underline{C}$H$_3$), 66.47 ($\underline{C}$HCH$_3$), 74.50 (OCH$_2$), 76.71 (NCH$_2$), 119.70 (C-5 imidazole), 145.38 (C imidazole), 146.52 (C imidazole). MS (EI) *m/z* (%) 215 M$^+$ (7), 171 (9), 141 (43), 127 (24), 89 (12), 59 (100), 43 (49), 28 (18).

2-Methyl-4-nitro-1-[(2-trifluoromethanesulphonyloxyprop-1-yloxy)methyl]imidazole (**2.90**)

This compound was prepared from 1-[(2-hydroxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.89** (15 mg, 0.07 mmol) and triflic anhydride (19 µl) and worked up according to the procedure used for the preparation and work-up of 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85**. The target compound 2-methyl-4-nitro-1-[(2-trifluoromethanesulphonyloxyprop-1-yloxy)methyl]imidazole **2.90** was obtained as an oil (24 mg, 100%). It was immediately dissolved in dry dichloromethane (1.2 ml), and refrigerated at -10°C.

1-[(2-Iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.92**)

A solution of of 1-[(2-hydroxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.89** (12 mg, 0.056 mmol) in dry acetonitrile (0.24 ml) was treated with neat triflic anhydride (18 µl, 0.1 mmol) at -10°C. The mixture was refrigerated for 2.5 hours at -10°C. The solvent was evaporated at room temperature with a stream of nitrogen and the residue dissolved in acetone (0.5 ml). A solution of sodium iodide (10 mg, 0.067 mmol) in acetone (0.34 ml) was added to the solution of the residue in acetone, and the reaction mixture was stirred at room temperature for 20 minutes. The reaction product was chromatographed over silica gel (3 g), using ethyl acetate/hexane/chloroform (70:15:15) as mobile phase. Pure fractions were combined and

evaporated to dryness. The residue was dissolved in dichloromethane (5 ml), the solution washed with sodium carbonate solution (1%, 2 ml), dried over anhydrous $MgSO_4$, and filtered. The filtrate was filtered through a short column of aluminium oxide to remove residual traces of triflic acid. The filtrate was evaporated to dryness to give 1-[(2-iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92** as an oil (20 mg, 100%) in 95% isomeric purity. (HPLC Method 6). ^{1}H NMR (300 MHz) δ: 1.87 (d, J = 7.0 Hz, ICHCH_3, 3H), 2.55 (s, CH_3 imidazole, 3H), 3.57-3.70 (m, OCH_2CH, 2H), 4.11-4.22 (m, CH_2CHI, 1H), 5.4 (s, NCH_2, 2H), 7.83 (s, *H* imidazole, 1H). ^{13}C NMR (75 MHz) δ: 13.05 (imidazole $\underline{C}H_3$), 20.96 ($CH\underline{C}H_3$), 24.31 (I-$\underline{C}HCH_3$), 76.21, 76.22 (NCH_2 and OCH_2), 119.62 (C-5 imidazole), 145.67 (C imidazole), 146.85 (C imidazole). MS (EI) *m/z* (%) 325 M^+ (11), 281 (8), 221 (12), 199 (20), 169 (100), 97 (12), 73 (22), 41 (73).

1,3-Dibenzoyloxy-2-propanol (**2.59**)

Method 1: A mixture of benzoic acid (1.76 g, 14.4 mmol) and sodium hydride (55-65% dispersion in oil[7], 1.0 g, 23-27 mmol) in HMPA (30 ml) was stirred at room temperature for 30 minutes, followed by the addition of a solution of 1,3-dibromo-2-propanol (1.5 g, 6.88 mmol) in HMPA (5 ml). The mixture was stirred in an oil bath at 110°C for 2.5 hours during which time its colour turned dark. The reaction mixture was cooled, a 5% HCl solution (80 ml) was added slowly to the mixture, and the mixture was extracted with diethyl ether (50 ml). The ethereal extract was washed with sodium hydrogen carbonate solution (5%, 30 ml), followed by water (30 ml), dried over anhydrous $MgSO_4$, filtered, and the filtrate evaporated to dryness to give a crude product containing 1,3-dibenzoyloxy-2-propanol **2.59** (1.67 g).

Method 2 : A mixture of sodium benzoate (2.08 g, 14.4 mmol) and 1,3-dibromo-2-propanol (1.5 g, 6.88 mmol) in DMF (50 ml) was stirred in an oil bath at 130-135°C for 1.5 hours. Additional sodium benzoate (1.0 g) was added to the yellow solution and the temperature was gradually increased to 165°C to achieve complete dissolution of the solid material. Heating was continued for another 20 minutes, after which the warm reaction mixture was filtered to remove unreacted sodium benzoate. The filtrate was concentrated to near dryness at 100°C

[7] The dispersion was not pre-washed to remove the oil

under reduced pressure. $CHCl_3$ (50 ml) and $NaHCO_3$ solution (5%, 40 ml) were added to the residue and the mixture well shaken in a separating funnel. The $CHCl_3$ layer was washed with water (40 ml), dried over anhydrous $MgSO_4$, the drying agent filtered off, and the filtrate evaporated to dryness to give a crude product containing 1,3-dibenzoyloxy-2-propanol **2.59** (1.74 g).

The crude extracts obtained from the two methods were combined and chromatographed over silica gel (30 g), using petroleum ether/ethyl acetate (2:1) as mobile phase. Fractions highly enriched in the target compound **2.59** were combined to give 1,3-dibenzoyloxy-2-propanol **2.59** as an oil (985 mg, 24%) in a purity of 95% (HPLC Method 4). ^{1}H NMR (300 MHz) δ: 2.91 (d, J = 4.9 Hz, O*H*, 1H), 4.38-4.44 (m, C*H*OH, 1H), 4.49-4.59 (ABX spin system, dd, $^2J_{gem}$ = 11.7 Hz, $^3J_{vic}$ = 5.3 Hz, $^3J_{vic}$ = 4.9 Hz, CH_2OCO (2), 4H), 7.45-7.50 (m, *H*-3, *H*-5 phenyl (2), 4H), 7.59-7.64 (m, *H*-4 phenyl (2), 2H), 8.08-8.11 (m, *H*-2, *H*-6 phenyl (2), 4H). ^{13}C NMR (75 MHz) δ: 65.82 ($CO_2\underline{C}H_2$), 68.53 (CHOH), 128.64 (phenyl C), 129.74 (phenyl C), 129.92 (phenyl C), 133.52 (phenyl C), 166.99 (C=O). MS (EI) *m/z* (%) 227 (2), 165 (13), 122 (8), 105 (100), 77 (31), 51 (10), 18 (15).

1,3-Dibenzoyloxy-2-chloromethoxypropane (**2.61**)

This reagent was prepared from 1,3-dibenzoyloxy-2-propanol **2.59** (313 mg, 1.0 mmol) and paraformaldehyde (37 mg, 1.23 mmol) according to the procedure used for the preparation of 2-chloromethoxyethyl benzoate **2.51**, except for allowing a slightly longer reaction time (20 minutes after introduction of **2.59**) and using a slightly higher temperature (-5 to -8°C). 1,3-Dibenzoyloxy-2-chloromethoxypropane **2.61** was obtained in a dichloromethane solution that was concentrated to a volume of 5 ml.

1-[(1,3-Dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.93**)

This compound was prepared from 2-methyl-5-nitroimidazole **2.79** (95 mg, 0.75 mmol), anhydrous K_2CO_3 (285 mg) and 1,3-dibenzoyloxy-2-chloromethoxypropane **2.61** in dichloromethane (5 ml, ~1 mmol based on the 1,3-dibenzoyloxy-2-propanol **2.59** content)

according to the procedure used for the preparation of 1-[(2-benzoyloxyprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.88**. Repeated column chromatography (twice) over silica gel (20 g, 8 g), using petroleum ether/ethyl acetate (2:1) and ethyl acetate as mobile phases for each procedure, gave 1-[(1,3-dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.93** (92 mg, 28%) in a purity of 96% (HPLC, Method 5). ^{1}H NMR (300 MHz) δ: 2.45 (s, C*H*$_3$ imidazole, 3H), 4.18-4.25 (m, OC*H*(CH$_2$)$_2$), 1H), 4.45-4.52 (dd, $^2J_{gem}$ = 12.1 Hz, $^3J_{vic}$ = 6.1 Hz, C*H*HOCO, 1H), 4.55-4.61 (dd, $^2J_{gem}$ = 12.1 Hz, $^3J_{vic}$ = 4.4 Hz, CH*H*OCO, 1H), 5.52 (s, NC*H*$_2$, 2H), 7.47-7.52 (t, *J* = 7.6 Hz, *H*-3, *H*-5 phenyl (2), 4H), 7.61-7.67 (t, *J* = 7.5 Hz, *H*-4 phenyl (2), 2H), 7.86 (s, *H* imidazole, 1H), 7.98-8.02 (m, *H*-2, *H*-6 phenyl (2), 4H). ^{13}C NMR (75 MHz) δ: 12.74 (imidazole C̲H$_3$), 63.16 (CO$_2$C̲H$_2$), 74.77 (CH-O), 75.21 (NCH$_2$), 119.72 (C-5 imidazole), 128.77 (phenyl C), 129.13 (phenyl C), 129.69 (phenyl C), 133.83 (phenyl C), 145.54 (C imidazole), 146.82 (C imidazole), 166.42 (C=O). MS (EI) *m/z* (%) 439 M$^+$ (1), 409 (0.5), 313 (13), 283 (9), 195 (3), 141 (7), 105 (100), 77 (15), 43 (3).

1-[(1-Benzoyloxy-3-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.94**)

A solution of concentrated ammonia (25%, 0.9 ml, ~13 mmol) was added to a solution of 1-[(1,3-dibenzoyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.93** (122 mg, 0.27 mmol) in methanol (9 ml) and the mixture was stirred at room temperature for 3 hours. The reaction mixture was diluted with water (9 ml), chloroform (15 ml) was added, and the mixture was vigorously stirred for 5 minutes. The layers were separated, and the aqueous layer was again extracted with $CHCl_3$ (5 ml). The combined organic extracts were dried over anhydrous $MgSO_4$, the drying agent filtered off, and the filtrate evaporated to dryness. The residue was chromatographed over silica gel (7 g), using ethyl acetate and ethyl acetate/methanol (20:1) as mobile phases, to give 1-[(1-benzoyloxy-3-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.94** (48 mg, 57%) in a purity of 96% [HPLC Method 2(b)]. ^{1}H NMR (300 MHz) δ: 2.48 (s, C*H*$_3$ imidazole, 3H), 3.77-3.83 (dd, $^2J_{gem}$ = 11.9 Hz, $^3J_{vic}$ = 6.5 Hz, C*H*HOH, 1H), 3.84-3.89 (dd, $^2J_{gem}$ = 11.8 Hz, $^3J_{vic}$ = 4.0 Hz, CH*H*OH, 1H), 3.94-4.0 (m, OC*H*(CH$_2$)$_2$, 1H), 4.36-4.42 (dd, $^2J_{gem}$ = 12.1 Hz, $^3J_{vic}$ = 6.0 Hz, C*H*HOCO, 1H), 4.44-4.5 (dd, $^2J_{gem}$ = 12.1 Hz, $^3J_{vic}$ = 4.2 Hz, CH*H*OCO, 1H), 5.51, 5.58 (AB spin system, $^2J_{gem}$ = 10.9 Hz, NC*H*$_2$, 2H),

7.47-7.53 (t, J = 7.6 Hz, *H*-3, *H*-5 phenyl, 2H), 7.61-7.67 (t, J = 7.5 Hz, *H*-4 phenyl, 1H), 7.88 (s, *H* imidazole, 1H), 7.97-8.0 (m, *H*-2, *H*-6 phenyl, 2H). ^{13}C NMR (75 MHz) δ: 12.80 (imidazole $\underline{C}H_3$), 62.57 (CH_2OH), 63.25 ($CO_2\underline{C}H_2$), 75.69 (NCH_2), 77.48 (CH-O), 119.96 (C-5 imidazole), 128.78 (phenyl C), 129.29 (phenyl C), 129.69 (phenyl C), 133.79 (phenyl C), 145.69 (C imidazole), 146.69 (C imidazole), 166.66 (C=O). MS (EI) *m/z* (%) 335 M^+ (1), 318 (1), 209 (13), 179 (6), 141 (7), 105 (100), 77 (18), 43 (8), 31 (2).

1-[(1-Benzoyloxy-3-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitro-imidazole (**2.95**) and *1-[(1-benzoyloxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole* (**2.99**)

A solution of 1-[(1-benzoyloxy-3-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.94** (23 mg, 0.068 mmol) in dry acetonitrile (0.5 ml) was treated with neat triflic anhydride (18 µl, 0.1 mmol) at -10°C. The mixture was refrigerated for 30 minutes at -10°C, the solvent evaporated with a stream of nitrogen at room temperature and the intermediate 1-[(1-benzoyloxy-3-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.95** dissolved in dry acetone (0.9 ml). This solution was treated with a solution of sodium iodide (18 mg, 0.12 mmol) in dry acetone (0.6 ml) and the mixture was stirred at room temperature for 1 hour. The acetone was evaporated with a stream of nitrogen, the reaction product dissolved in $CHCl_3$ (2 ml) and washed with sodium hydrogen carbonate (1%, 2 ml). The organic layer was dried over anhydrous $MgSO_4$, the drying agent filtered off, and the product chromatographed over silica gel (3 g), using $CHCl_3$ and then ethyl acetate as mobile phases, giving 1-[(1-benzoyloxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.99** (15 mg, 50% based on **2.94**). ^{1}H NMR (300 MHz) δ: 2.53 (s, CH_3 imidazole, 3H), 3.22-3.28 (dd, $^2J_{gem}$ = 11.1 Hz, $^3J_{vic}$ = 6.9 Hz, C*H*HI, 1H), 3.30-3.35 (dd, $^2J_{gem}$ = 11.2 Hz, $^3J_{vic}$ = 4.7 Hz, CH*H*I, 1H), 3.89-3.97 (m, OC*H*$(CH_2)_2$, 1H), 4.37-4.43 (dd, $^2J_{gem}$ = 12.1 Hz, $^3J_{vic}$ = 5.8 Hz, C*H*HOCO, 1H), 4.47-4.53 (dd, $^2J_{gem}$ = 12.1 Hz, $^3J_{vic}$ = 4.5 Hz, CH*H*OCO, 1H), 5.48 (s, NCH_2, 2H), 7.47-7.53 (CC'-component of AA'BCC' spin system, *H*-3, *H*-5 phenyl, 2H), 7.62-7.67 (B-component of AA'BCC' spin system, *H*-4 phenyl, 1H), 7.85 (s, *H* imidazole, 1H), 7.98-8.02 (AA'-component of AA'BCC' spin system, *H*-2, *H*-6 phenyl, 2H). ^{13}C NMR (75 MHz) δ: 2.44

(CH_2I), 13.06 (imidazole $\underline{C}H_3$), 65.07 ($CO_2\underline{C}H_2$), 75.30 (NCH_2), 76.73 (CH-O), 119.77 (C-5 imidazole), 128.84 (phenyl C), 129.05 (phenyl C), 129.74 (phenyl C), 133.95 (phenyl C), 145.76 (C imidazole), 146.85 (C imidazole), 166.37 (C=O). MS (EI) *m/z* (%) 445 M^+ (1), 318 (12), 289 (34), 232 (2), 191 (3), 149 (2), 105 (100), 77 (17), 43 (22).

*1-[(1-Benzoyloxy-3-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole* (**2.97**)

A mixture of 1-[(1-benzoyloxy-3-hydroxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.94** (21 mg, 0.06 mmol) and *p*-toluenesulphonyl chloride (36 mg, 0.19 mmol) in dry pyridine (1 ml) was stirred at room temperature for 4 hours and then refrigerated overnight at -10°C. The reaction mixture was diluted with ethyl acetate (3 ml), and washed twice with water (3 ml). The combined aqueous phase was extracted with ethyl acetate (3 ml), and the combined organic extracts dried over anhydrous $MgSO_4$. The drying agent was filtered off, and the solvent evaporated at 70°C under reduced pressure. The residue was chromatographed over silica gel (6 g), using petroleum ether/ethyl acetate (2:1), followed by ethyl acetate, as mobile phases. Fractions showing essentially only one spot were combined, evaporated to dryness, dissolved in $CHCl_3$ (4 ml), and washed twice with 0.1 N H_2SO_4 (4 ml) to remove traces of pyridine. The $CHCl_3$ layer was filtered through a pad of anhydrous $MgSO_4$ and the filtrate evaporated to dryness. The residue was finally dried in vacuum at 60°C to give 1-[(1-benzoyloxy-3-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.97** (24 mg, 79%). ^{1}H NMR (300 MHz) δ: 2.45 (2 s, C*H*$_3$ imidazole and Ts-C*H*$_3$ overlap, 6H), 4.06-4.43 (m, OC*H*$_2$C*H*C*H*$_2$O overlap, 5H), 5.45 (s, NC*H*$_2$, 2H), 7.36-7.39 (m, BB'-component of AA'BB' spin system, Ts aromatic *H*, 2H), 7.46-7.52 (CC'-component of AA'BCC' spin system, *H*-3, *H*-5 phenyl, 2H), 7.62-7.67 (B-component of AA'BCC' spin system, *H*-4 phenyl, 1H), 7.79-7.81 (m, *H* imidazole, AA'-component of AA'BB' spin system, Ts aromatic *H* overlap, 3H), 7.92-7.95 (AA'-component of AA'BCC' spin system, *H*-2, *H*-6 phenyl, 2H). ^{13}C NMR (75 MHz) δ: 12.74 (imidazole $\underline{C}H_3$), 21.46 (Ts-$\underline{C}H_3$), 62.25 ($CO_2\underline{C}H_2$), 68.43 ($\underline{C}H_2$OTs), 74.54 (NCH_2), 75.52 (CH-O), 119.70 (C-5 imidazole), 128.00 (Ts aromatic C), 128.79 (phenyl C), 128.92 (phenyl C), 129.70 (phenyl C), 130.28 (Ts aromatic C), 132.27 (Ts aromatic C), 133.93

(phenyl C), 145.62 (C imidazole), 145.92 (Ts aromatic C), 146.87 (C imidazole), 166.22 (C=O). MS (EI) *m/z* (%) 489 M^+ (1), 363 (3), 333 (9), 177 (1), 155 (11), 105 (100), 91 (22), 77 (20), 18 (8).

1-[(1-Hydroxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole (**2.100**)

A solution of sodium hydroxide (1 N, 0.15 ml, 0.15 mmol) was mixed with a solution of 1-[(1-benzoyloxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.99** (12 mg, 0.027 mmol) in methanol (1.2 ml) and the mixture was stirred at room temperature for 50 minutes. The methanol was evaporated with a stream of nitrogen, water (1 ml) and $CHCl_3$ (2 ml) was added to the residue, and the mixture was stirred for 5 minutes. The layers were separated, the aqueous phase extracted twice with $CHCl_3$ (1 ml) and the combined $CHCl_3$ extracts dried over anhydrous $MgSO_4$. The drying agent was filtered off, and the filtrate evaporated to dryness. The filtrate residue was chromatographed over silica gel (3 g), using ethyl acetate as mobile phase, to give 1-[(1-hydroxy-3-iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100** (6.5 mg, 70%). ^{1}H NMR (600 MHz) δ: 2.55 (s, C*H*$_3$ imidazole, 3H), 3.13-3.16 (dd, $^2J_{gem}$ = 11.0 Hz, $^3J_{vic}$ = 6.6 Hz, C*H*HI, 1H), 3.19-3.22 (dd, $^2J_{gem}$ = 11.0 Hz, $^3J_{vic}$ = 4.6 Hz, CH*H*I, 1H), 3.66-3.81 (m, C*H*$_2$OH, OC*H*(CH$_2$)$_2$) overlap, 3H), 5.47, 5.54 (AB spin system, dd, $^2J_{gem}$ = 11.0 Hz, NC*H*$_2$, 2H), 7.84 (s, *H* imidazole, 1H). ^{13}C NMR (150 MHz) δ: 2.88 (CH$_2$I), 13.32 (imidazole CH$_3$), 65.01 (CH$_2$OH), 75.70 (NCH$_2$), 79.18 (CH-O), 119.84 (C-5 imidazole), 145.65 (C imidazole), 146.53 (C imidazole). MS (EI) *m/z* (%) 341 M^+ (6), 214 (10), 185 (100), 184 (63), 128 (24), 111 (7), 87 (43), 57 (79), 29 (40).

6.4 RADIOSYNTHESES

Radioactivity quantities are all given within ranges due to the varying radioactivity concentrations of the radioiodine sources.

6.4.1 General Method for the Radiosynthesis of β-Iodoalkylether Model Compounds **2.4**, **2.7**, **2.12**, **2.15**, **2.19**, **2.22** and Reference Compounds **2.26** and **2.30**

A radioiodide solution [0.5-5.0 µl, 30-100 µCi (1.1-3.7 MBq) ^{131}I] and a solution of sodium sulphite (0.5 mg) in water (20 µl) were placed in a 5 ml pear-shaped vessel or a 2 ml ReactiVial with a screw cap. The mixture was evaporated to dryness with a stream of argon or nitrogen with mild heating. A solution of the respective tosylate (5-10 mg) [30-50 mg in the case of the tosylate 2-methoxy-2-phenylethyl *p*-toluenesulphonate **2.21**] in acetone/dioxane (4:1) or acetone (0.5 ml) was added to the dried mixture, the vessel fitted with a reflux condenser (or the vial was sealed) and the mixture was heated and stirred in an oil bath at 120-180°C. The reaction progress was monitored by means of radio-thin layer chromatography (radio-TLC), using Method 1 and autoradiography as imaging tool. Small amounts of the corresponding cold iodinated compounds were used as reference compounds to distinguish the authentic radioiodinated compounds from radiochemical impurities. Some reactions were also monitored by means of radio-HPLC, using Method 8 or 9. After completion of the reaction, indicated by the consumption of most of the radioiodide and the formation of the desired radioiodinated compound, the volume of the reaction mixture was reduced to approximately 0.2 ml with a slow stream of argon or nitrogen. Hexane (1 ml) was added and the mixture was transferred to a silica gel column (3 - 4g silica gel equilibrated with hexane/chloroform (90:10), column diameter = 1-1.5 cm). Fractions were eluted with hexane/$CHCl_3$ (90:10) or (70:30). The purities of these fractions were determined by means of autoradiography as described above. Pure fractions were combined, evaporated to dryness under reduced pressure at approximately 20°C, and the residue dissolved in a small amount of human serum (50-90 µl) by means of a strong vortex action.

6.4.2 General Method for the Radiosynthesis of Benzamide Derivatives **2.42**, **2.44**, **2.46** and **2.48**

6.4.2.1 From tosylate precursors 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)ethoxy]benzamide **2.41**, 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[1-(*p*-toluenesulphonyloxy)prop-2-yloxy]benzamide **2.43** and 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[2-(*p*-toluenesulphonyloxy)prop-1-yloxy]-benzamide **2.45**

A radioiodide solution [50 µl, 12-19 mCi (444-703 MBq) ^{123}I] was placed in a 1 ml ReactiVial and evaporated to dryness with a stream of nitrogen with mild heating. A solution of the tosylate (1 mg) in acetone (0.1 ml) was added to the radioiodide, the vial was capped and heated in an oil bath at a temperature of 150°C (150-170°C for tosylate **2.45**) for 15-20 minutes. The vial was cooled, a small aliquot was taken for HPLC analysis (Method 1), and the acetone was evaporated with a stream of nitrogen. The residue was dissolved in diisopropyl ether/methanol/25% ammonia (85:15:0.2) (0.3 ml) and transferred to a small (~1 cm diameter) silica gel column (2 g silica gel equilibrated with the same solvent mixture). Residual activity was washed into the column (0.3 ml), and the product was eluted with diisopropyl ether/methanol/25% aqueous ammonia (85:15:0.2). Approximately ten fractions of 2 ml each were collected and the activity in each fraction was measured. Fractions containing the highest activities were combined, and evaporated to dryness with a stream of nitrogen. The resulting residue was dissolved in a small amount of serum (90 µl) as described previously.

6.4.2.2 Radiosynthesis of 2-(3-[^{123}I]iodoprop-2-en-1-yloxy)-3-methoxy-*N*-(2-piperidin-1-ylethyl)benzamide **2.48** from precursor 3-methoxy-*N*-(2-piperidin-1-ylethyl)-2-[3-(tributylstannyl)prop-2-en-1-yloxy]benzamide **2.47**

A radioiodide solution [50 µl, 14 mCi (518 MBq) ^{123}I] and a solution of precursor **2.47** (1 mg) in acetonitrile (195 µl) were placed in a 10 ml borosilicate glass vial. A solution of H_2SO_4

(10%, 105 µl), and a solution of chloramine-T[8] (30 µg) in water (30 µl) was added to the contents in the vial and the mixture was stirred for 5 minutes at room temperature. The reaction was quenched with a solution of sodium metabisulphite (145 µg) in water (145 µl), the reaction mixture neutralized to a pH of 6-7 with a solution of sodium carbonate (30 mg) in water (1 ml), and extracted with dichloromethane (3 ml). The dichloromethane extract was evaporated to dryness with a stream of nitrogen, and the residue dissolved in a mixture of diisopropyl ether/methanol/25% ammonia (70:30:0.5) (0.2 ml). The solution was transferred to a silica gel column prepared as before and the product was eluted with diisopropyl ether/methanol/25% ammonia (70:30:0.5). Ten fractions of 1 ml each were collected and the activity in each fraction was measured. Fractions containing the highest activities were combined, and evaporated to dryness with a stream of nitrogen. The residue was dissolved in a small amount of serum (90 µl) as previously described.

6.4.3 General Methods for the Radiosynthesis of Heterocyclic Amine Derivatives

6.4.3.1 Radiosynthesis of benzotriazole derivatives 1-[(2-[^{123}I]iodoethoxy)methyl]benzotriazole **2.66b** and 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]benzotriazole **2.70**

A radioiodide solution [50 µl, 15-19 mCi (555-703 MBq] was placed in a 1 ml ReactiVial and evaporated to dryness with a stream of nitrogen with mild heating. A solution of the respective tosylates 1-[(2-*p*-toluenesulphonyloxyethoxy)methyl]benzotriazole **2.65** and 1-[(1-*p*-toluenesulphonyl-oxyprop-2-yloxy)methyl]benzotriazole **2.69** (1 mg) in acetone (0.1 ml) was added to the radioiodide, the vial was capped and heated for 10 minutes in an oil bath at a temperature of 130°C (for the radiosynthesis of **2.66b**) or 130°-150°C (for the radiosynthesis of **2.70**). The vial was cooled, a small aliquot was taken for HPLC analysis [Method 2(a) or 2(b)], and the reaction mixture diluted with petroleum ether (0.3 ml). The mixture was transferred to a small column of silica gel [2 g, equilibrated with petroleum ether/ethyl acetate (5:1)]. The reaction vial was rinsed with the same solvent mixture (0.3 ml), the liquid transferred to the

[8] The sodium salt of *N*-chloro-*p*-toluenesulphonamide

column and the product eluted with petroleum ether/ethyl acetate (5:1). Thirteen fractions of 2 ml each were collected, and the activity in each fraction was measured. Fractions containing the highest activities were combined, and evaporated to dryness with a stream of nitrogen. The residue was dissolved in a small amount of serum (90 µl) as previously described.

6.4.3.2 Radiosynthesis of Benzotriazole derivatives 1-(2-[^{123}I]iodoprop-1-yl)benzotriazole **2.74** and 1-(3-[^{123}I]iodo-2-methylprop-1-yl)benzotriazole **2.78**

The radiosynthesis and purification of these compounds were carried out according to the procedure used for the preparation of [^{123}I]**2.66b** and [^{123}I]**2.70**, except for using an oil bath temperature of 150°C, and petroleum ether/ethyl acetate (3:1) as mobile phase for the chromatography over silica gel.

6.4.3.3 Radiosynthesis of 2-methyl-4-nitroimidazole derivative 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87**

6.4.3.3.1 From 2-methyl-4-nitro-1-[(1-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]imidazole **2.83**

The radiosynthesis and purification of [^{123}I]**2.87**, using the tosylate precursor **2.83**, was carried out according to the procedure used for the preparation of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]benzotriazole **2.70**, except for the following differences:
Volume and activity of radioiodide solution: 65 µl, 19-20 mCi (703-740 MBq).
Oil bath temperature: 130°C.
Mobile phase for chromatography over silica gel: Ethyl acetate/hexane/$CHCl_3$ (70:15:15).
Fraction volumes: 1 ml (Nine fractions).

6.4.3.3.2 From 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85**

A radioiodide solution [50 µl, 11-14 mCi (407-518 MBq) ^{123}I] was placed in a 10 ml borosilicate glass vial and the solution was evaporated to dryness with a stream of nitrogen with mild heating. A solution of the triflate **2.85** (1-5 mg) in dichloromethane (50-250 µl) was dried with a stream of nitrogen, the dried triflate re-dissolved in dry acetone (200 µl) and the solution transferred to the vial containing the dried radioiodide. The mixture was stirred at room temperature for 10-30 minutes. A small aliquot was taken for HPLC analysis [Method 2(b], the reaction mixture diluted with the mobile phase, ethyl acetate/hexane/$CHCl_3$ (70:15:15) (0.3 ml), and chromatographed over a small silica gel column equilibrated with the mobile phase. Ten 1 ml fractions were collected. Fractions containing the highest activities were combined, evaporated and the residue dissolved in serum as before.

6.4.3.4 Radiosynthesis of 2-methyl-4-nitroimidazole derivative 1-[(2-[^{123}I]iodoprop-1-yloxy)methyl]-2-methyl-4-nitroimidazole **2.92**

The radiosynthesis and purification of [^{123}I]**2.92** from 2-methyl-4-nitro-1-[(2-trifluoromethanesulphonyloxyprop-1-yloxy)methyl]imidazole **2.90** was carried out according to the procedure used for the preparation of 1-[(1-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.87** from 2-methyl-4-nitro-1-[(1-trifluoromethanesulphonyloxyprop-2-yloxy)methyl]imidazole **2.85**, except for the following differences:

Volume and activity of radioiodide solution: 30-75 µl, 7-22 mCi (259-814 MBq).
Mass of triflate used: 1-9 mg.

6.4.3.5 Radiosynthesis of 2-methyl-4-nitroimidazole derivative 1-[(1-hydroxy-3-[^{123}I]iodo-prop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.100** via 1-[(1-benzoyloxy-3-[^{123}I]iodoprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.99**

A radioiodide solution [50 µl, 14 mCi (518 MBq) ^{123}I] was placed in a 1 ml ReactiVial and evaporated to dryness with a stream of nitrogen with mild heating. A solution of the tosylate precursor 1-[(1-benzoyloxy-3-*p*-toluenesulphonyloxyprop-2-yloxy)methyl]-2-methyl-4-nitroimidazole **2.97** (1 mg) in acetone (0.1 ml) was added to the radioiodide, the vial was capped and heated in an oil bath at 150°C for 30 minutes. The vial was cooled, a small aliquot was taken for HPLC analysis [Method 2(b)], and the reaction mixture transferred with a small syringe to a 10 ml borosilicate glass vial. The reaction vial was rinsed with acetone (0.3 ml) and the liquid transferred to the 10 ml vial. The acetone solution was evaporated to dryness at room temperature with a stream of nitrogen, and the residue, containing [^{123}I]**2.99**, was dissolved in 96% ethanol (0.2 ml). Water (0.1 ml) and 1M NaOH (50 µl) were added to the ethanol solution, and the mixture was stirred at room temperature for approximately 30 minutes. The mixture was then filtered through a small column containing a small amount of a water-moistened cation exchange resin [AGMP-50 hydrogen form (BioRad), 200-400 mesh, approximately 100 mg]. The reaction vial was rinsed with ethanol (0.2 ml), and the liquid transferred to the resin column. The filtrate was evaporated to near dryness with a stream of nitrogen and mild heating, the residue dissolved in ethyl acetate (0.2 ml) and the solution transferred to a small silica gel (2 g) column, equilibrated with ethyl acetate. The product, [^{123}I]**2.100**, was eluted with ethyl acetate, and nine 2 ml fractions were collected. Fractions containing the highest activities were combined and evaporated to dryness under nitrogen with mild heating. The residue was dissolved in serum as before.

6.5 STABILITY EVALUATION

6.5.1 Stability Evaluation of the Labelled Pilot Study Compounds **2.4**, **2.7**, **2.12**, **2.15**, **2.19**, **2.22** and the Reference Compounds **2.26** and **2.30**

A small amount of the labelled compound (5 - 20 μCi, 0.185 - 0.74 MBq), dissolved in human blood serum (50-90 μl), was placed in a small Eppendorff vial or a 5 ml round bottom glass vessel and diluted further with blood serum (110 - 250 μl). An aliquot (30-45 μl) of the serum solution was taken and diluted with acetonitrile (40-60 μl) containing 40-45 μg of the corresponding cold iodinated compound. This aliquot represented the time 0 sample. The vial or vessel was sealed and placed in a waterbath or incubator at 37°C. Aliquots (30-45 μl) of the incubation mixture were taken after 3 and 21 hours incubation time and diluted with acetonitrile in the same way as for the time 0 sample. The diluted mixtures were thoroughly mixed by a vortex action, and the supernatent was analysed for free radioiodide. In most cases, both radio-HPLC and -TLC were used to determine the amount of released free radioiodide.

In the radio-HPLC analysis, an aliquot (15 - 30 μl) of the supernatent was injected into the HPLC, and the areas of the peaks processed on a chromatogram. Retention times were as follows:
Method 8: Free radioiodide, 2.1 - 2.4 min; labelled compound, 7 - 9 min.
Method 9: Free radioiodide, 1.4 – 1.6 min; labelled compound, 7.5 - 12 min.

In the radio-TLC analysis, a small aliquot (6 - 20 μl) of the supernatent solution was applied to a silica gel TLC plate in the presence of approximately 30 μg stable sodium iodide. The plate was developed by using TLC Method 12. The plate was then allowed to dry and imaged by means of autoradiography. The intensities of the spots were automatically processed and expressed as area percentages in the same way as in an HPLC chromatogram. The relative retention factors were as follows:
Free radioiodide, 0.4-0.5; labelled compound, 1.

The amount of free radioiodide was defined as the area of the radioactive component associated with free radioiodide expressed as a percentage of the total area associated with free radioiodide as well as that associated with the labelled compound displayed in a chromatogram.

6.5.2 Stability Evaluation of the Labelled Benzamide and Heterocyclic Amine Derivatives **2.42**, **2.44**, **2.46**, **2.48**, **2.66b**, **2.70**, **2.74**, **2.78**, **2.87**, **2.92** and **2.100**

A small amount of the labelled compound (0.5 - 2 mCi, 18.5 - 74 MBq), dissolved in human blood serum (80 µl), was placed in a 5 ml round bottom glass vessel and diluted further with blood serum (220 or 320 µl). An aliquot (30 µl) of the serum solution was taken and diluted with acetonitrile (60 µl). This aliquot represented the time 0 sample. The vessel was sealed and placed in an incubator oven at 37°C. Aliquots (30 µl) of the incubation mixture were taken after 2, 5 and 24 hours incubation time and diluted with acetonitrile in the same way as for the time 0 sample. The diluted mixtures were thoroughly mixed by a vortex action, and the supernatent liquid was analysed for free radioiodide by means of HPLC. An aliquot of the supernatent solution (5-20 µl) was co-injected with a small amount of stable sodium iodide (40 µg).

In the analysis of the labelled benzamide derivatives, HPLC Method 1 was used. The retention times of the various compounds were as follows:
Free radioiodide, 2.0 - 2.2 min.
Labelled compound **2.42**, 12.8 - 13.4 min.
2.44, 14.2 - 14.8 min.
2.46, 14.2 - 15.5 min.
2.48, 14.6 - 15.3 min.

In the analyses of the labelled heterocyclic amine derivatives, both Methods 2(a) and 2(b) were used for labelled compounds **2.66b** and **2.70**, while only Method 2(b) was used for the other

compounds in this series. No differences in retention times were observed when using these two methods. The following retention times were recorded for the various compounds:

Free radioiodide, 2.0 - 3.0 min.

Labelled compound **2.66b**, 15.1 - 16.2 min.

2.70, 17.6 - 18.3 min.

2.74, 16.5 - 16.9 min.

2.78, 18.9 - 19.1 min.

2.87, 12.4 - 12.9 min.

2.92, 13.2 - 13.9 min.

2.100, 6.0 - 6.5 min.

The amount of free radioiodide was defined in the same way as described in section 6.5.1.

APPENDIX A

CHROMATOGRAPHIC PARAMETERS FOR THE BENZAMIDE DERIVATIVES

Compound No.	HPLC Method No.	HPLC Retention Time (Rt) (min.)	TLC Method No.	TLC Retention Factor (Rf) (min.)
2.33	1	2.4	5	0.1
2.41	1	12.2		-
2.42	1	13.3		-
2.43	1	16.1	5	0.48
2.44	1	14.8	5	0.66
2.45	1	15.0	5	0.37
2.46	1	14.8	5	0.57
2.47		-	4	0.75
2.48	1	14.6	4	0.53

APPENDIX B

CHROMATOGRAPHIC PARAMETERS FOR THE BENZOTRIAZOLE DERIVATIVES

Compound No.	HPLC Method No.	HPLC Retention Time (Rt) (min.)	TLC Method No.	TLC Retention Factor (Rf) (min.)
2.62	2b	4.8	6	0.63
2.63	2b	17.5	6	0.75
2.64	2b	4.2	6	0.15
2.64	6	14.3		
2.65	2a	17.8	6	0.61
2.66a	2b	12.2	-	-
2.66b	2b	15.4	-	-
2.67	2b	18.8	7	0.51
2.68	2b	5.2	6	0.31
2.68	6	15.7		
2.69	2b	19.0	6	0.63
2.70	2b	18.0	7	0.63
2.71	7	7.5	6	0.37
2.72	7	12.5	6	0.57
2.73	2b	17.0	7	0.37
2.74	2b	16.7	-	-
2.76	7	12.0	6	0.34
2.77	2b	19.1	7	0.46
2.78	2b	18.9	7	0.62

APPENDIX C

CHROMATOGRAPHIC PARAMETERS FOR THE 2-METHYL-4-NITROIMIDAZOLE DERIVATIVES

Compound No.	HPLC Method No.	HPLC Retention Time (Rt) (min.)	TLC Method No.	TLC Retention Factor (Rf) (min.)
2.79	5	2.9	-	-
2.80	5	8.5	9	0.67
2.80	4	13.6		
2.82	5	3.1	9	0.2
2.82	6	10.9		
2.83	2b	16.2	10	0.45
2.85	-	-	11	0.66
2.87	2b	12.5	-	-
2.88	5	7.6	9	0.71
2.88	4	15.3		
2.89	4	3.1	11	0.24
2.89	6	10.1		
2.90	-	-	11	0.66
2.92	2b	13.3	-	-
2.93	5	13.5	9	0.73
2-Methyl-5-nitro-imidazole derivative of **2.93**	5	14.6	9	0.58
2.94	2b	9.5	11	0.36
2.95	2b	19.5	9	0.74
2.97	-	-	9	0.68
2.99	2b	18.3	9	0.66
2.100	2b	6.0	9	0.37

REFERENCES

Abbadi M., Mathieu J-P. and Morin C. (1997). A synthesis of 4-deoxy-4-iodo-D-glucose suitable for radiolabelling. *Journal of Labelled Compounds and Radiopharmaceuticals*, **39**, 487-492.

Anders M.W. and Pohl L.R. (1985). Toxicity of halogenated alkanes. In: Bioactivation of Foreign Compounds, Edited by M.W. Anders, Academic Press, Orlando, Florida, Chapter 10, p. 283-315.

Andrews G.A. (1976). History of Radiopharmacy. In: Radiopharmy, Edited by M. Tubis and W. Wolf, Publ. John Wiley & Sons, Inc., p. 3.

Argentini M., Zahner M. and Schubiger P.A. (1981). Comparison of several methods for the synthesis of ω-iodine-123-heptadecanoic acid. *Journal of Radioanalytical Chemistry*, **65**, 131-138.

Aşikoğlu M., Yurt F. and Ünak P. (1998). Labeling of ornidazole with iodine-131. *Journal of Radioanalytical and Nuclear Chemistry*, **237**, 143-145.

Asikoglu M., Yurt F., Cagliyan O., Unak P. and Ozkilic H. (2000). Detecting inflammation with ^{131}I-labeled ornidazole. *Applied Radiation and Isotopes*, **53**, 411-413.

Baumstark F. (1874). *Berichte der Deutschen Chemischen Gesellschaft*, **7**, 1172-1175 (Reference in: Hamant *et al.*, 1994).

Bignan G., Mathieu J-P., Mauclaire L., Morin C. and Vidal M. (1993). Synthesis of radiolabeled ω-iodoalkyl glucosides. *Journal of Labeled Compounds and Radiopharmaceuticals*, **32**, 584-585 [Symposium Abstracts].

Biskupiak J.E., Grierson J.R., Rasey J.S., Martin G.V. and Krohn K.A. (1991). Synthesis of an (iodovinyl)misonidazole derivative for hypoxia imaging. *Journal of Medicinal Chemistry,* **34**, 2165-2168.

Bobeldijk M., Verhoeff N.P.L.G., Vekemans J.A.J.M., Buck H.M., Van Doremalen P.A.P.M., Van Hoof J.J. and Janssen A.G.M. (1990). A simple and high-yield synthesis of (*S*)-BZM, (*R*)-BZM and (*S*)-IBZM for the preparation of (*S*)-^{123}I-IBZM. *Journal of Labeled Compounds and Radiopharmaceuticals*, **28**, 1247-1256.

Bolton A.E. and Hunter W.M. (1973). The labelling of proteins to high specific radioactivities by conjugation to a I-125-containing acylating agent. *Biochemistry Journal*, **133**, 529-539.

Bonini C., Giuliano C., Righi G. and Rossi L. (1992). An easy procedure for the highly regioselective conversion of epoxides to halohydrins. *Synthetic Communications*, **22**, 1863-1870.

Bonini C. and Righi G. (1994). Regio- and chemoselective synthesis of halohydrins by cleavage of oxiranes with metal halides. *Synthesis*, 225-238.

Canney D.J., Guo Y-Z., Kung M-P. and Kung H.F. (1993). Synthesis and preliminary evaluation of an iodovinyl-tetrabenazine analog as a marker for the vesicular monoamine transporter. *Journal of Labeled Compounds and Radiopharmaceuticals*, **33**, 355-368

Casper J. (1967). The introduction in 1928-29 of thorium dioxide in diagnostic radiology. *Annals of the New York Academy of Science*, **145**, 527-529.

Casy A.F. and Parfitt R.T. (1986). *Opioid Analgesics Chemistry and Receptors,* Plenum Publishing Corp., New York. (Reference in: Musachio and Lever, 1992).

Chalon S. Frangin Y., Guilloteau D., Caillet M., Guimbal C., Schmitt M-H., Desplanches G., Baulieu J-L. and Besnard J-C. (1990). Iodoethylspiperone, a new potential agent for exploration of central dopamine D_2 receptors: Synthesis and preliminary *in vivo* study. *International Journal of Radiation Applications and Instrumentation Part B. Nuclear Medicine and Biology*, **17**, 389-395.

Charronneau E., Mathieu J-P. and Morin C. (1998). Large-scale synthesis and radiolabelling of 6-deoxy-6-iodo-D-glucose (6-DIG). *Applied Radiation and Isotopes*, **49**, 1605-1607.

Chattopadhyay S., Das M.K., Sarkar B.R., Prabhakar G., Mehra K.S. and Ramamoorthy N. (2001). Stabilisation of [^{131}I]*meta*-iodobenzylguanidine at room temperature as organic extract in ethyl acetate/chloroform. *Applied Radiation and Isotopes*, **54**, 241-244.

Chenoweth M.B. and McCarty L.P. (1963). On the mechanisms of the pharmacophoric effect of halogenation. *Pharmacological Reviews*, **15**, 673-707.

Chi D.Y., Kilbourn M.R., Katzenellenbogen J.A., Brodack J.W. and Welch M.J. (1986). Synthesis of *N*-(ω-[^{18}F]fluoroalkyl)spiperone derivatives: Rapid and efficient methods for the [^{18}F]fluoroalkylation of amines and amides. *Journal of Labeled Compounds and Radiopharmaceuticals*, **23**, 1035-1037 [Symposium Abstracts].

Chumpradit S., Kung M-P., Vessotskie J. and Kung H.F. (1995). Synthesis, resolution and radioiodination of *S*(-)*trans*-5-hydroxy-2-[*N*-*n*-propyl-*N*-(3'-iodo-2'-propenyl)amino]tetralin-*S*(-)*trans*-5-OH-PIPAT: A new dopamine D2-like receptor ligand. *Journal of Labeled Compounds and Radiopharmaceuticals*, **36**, 1051-1062.

Clanton J.A., de Paulis T., Schmidt D.E., Ansari M.S., Manning R.G., Baldwin R.M. and Kessler R.M. (1991). Preparation of [^{123}I]- and [^{125}I]epidepride: A dopamine D-2 receptor

antagonist radioligand. *Journal of Labeled Compounds and Radiopharmaceuticals*, **29**, 745-751.

Coenen H.H., Moerlein S.M. and Stöcklin G. (1983). No-carrier-added radiohalogenation methods with heavy halogens. *Radiochimica Acta*, **34**, 47-68.

Dawe R.D., Molinski T.F. and Turner J.V. (1984). Dilithium tetrabromonickelate(II) as a source of soft nucleophilic bromide: Reaction with epoxides. *Tetrahedron Letters*, **25**, 2061-2064.

Demole E. (1876). *Berichte der Deutschen Chemischen Gesellschaft*, **9**, 743-747 (Reference in: Hamant *et al.*, 1994).

De Paulis T., Bingham D.R., Manning R.G., Mason N.S., Ansari M.S., Schmidt D.E. and Kessler R.M. (1991). Design and radiofluorination of benzamide dopamine D2 receptor ligands. In: New trends in Radiopharmaceutical Synthesis, Quality Assurance, and Regulatory Control, Edited by A.M. Emran, Plenum Press, New York, p. 97-107.

Dolbier W.R. (Jr.), Li A-R., Koch C.J., Shiue C-Y. and Kachur A.V. (2001). [^{18}F]-EF5, a marker for PET detection of hypoxia: synthesis of precursor and a new fluorination procedure. *Applied Radiation and Isotopes*, **54**, 73-80.

Eary J.F., Krohn K.A., Kishore R. and Nelp W.B. (1988). Radiochemistry of halogenated antibodies. In: Antibodies in radiodiagnosis and therapy, Edited by M.R. Zalutsky, CRC Press, New York (Reference in: Warren *et al.*, 1990).

Eersels J.L.H. and Herscheid J.D.M. (2001). *o*-Iodohippuric acid as stabilizer against deiodination of I-123-radiopharmaceuticals. *Journal of Labeled Compounds and Radiopharmaceuticals*, **44**, Suppl.1, S939-941.

Eisch J.J., Liu Z., Ma X. and Zheng G. (1992). High regioselectivity in the alternative, halogenative cleavages of terminal epoxides with Lewis acid metal halides. *Journal of Organic Chemistry*, **57**, 5140-5144.

Fleet G.W.J. (1989). Homochiral compounds from sugars. *Chemistry in Britain,* 287-292.

French A.N., Napolitano E., VanBrocklin H.F., Brodack J.W., Hanson R.N., Welch M.J. and Katzenellenbogen J.A. (1991). The β-heteroatom effect in metabolic defluorination: the interaction of resonance and inductive effects may be a fundamental determinant in the metabolic lability of fluorine-substituted compounds. *Journal of Labeled Compounds and Radiopharmaceuticals*, **30**, 431-433 [Symposium Abstracts].

French A.N., Napolitano E., VanBrocklin H.F., Hanson R.N., Welch M.J. and Katzenellenbogen J.A. (1993). Synthesis, radiolabeling and tissue distribution of 11β-fluoroalkyl- and 11β-fluoroalkoxy-substituted estrogens: Target tissue uptake selectivity and defluorination of a homologous series of fluorine-18-labeled estrogens. *Nuclear Medicine and Biology*, **20**, 31-47.

Furniss B.S., Hannaford A.J., Smith P.W.G. and Tatchell A.R. (1989). Vogel's Textbook of Practical Organic Chemistry, 5th edition, p. 1206-1207.

Guédin-Vuong D. and Nakatani Y. (1986). An easy access to homopropargylic ethers. *Bulletin de la Société Chimique de France*, 245-252.

Hakimelahi G.H., Zarrinehzad M., Jarrahpour A.A. and Sharghi H. (1987). Ring-open analogues of adenine nucleoside. Aminoacyl derivatives of cyclo- and acyclo-nucleosides. *Helvetica Chimica Acta*, **70**, 219-231.

Hamant S., Mathieu J-P., Morin C., Trimcev I. and Vidal M. (1994). The β-iodoethoxyl group: A stable unit for radioiodination. *Bioorganic and Medicinal Chemistry Letters*, **4**, 1687-1690.

Hanson R.N. and Seitz D.E. (1982). Radioiododestannylation: A method for specifically labeled radiopharmaceuticals. *Journal of Labeled Compounds and Radiopharmaceuticals*, **19**, 1585-1586.

Hanson R.N., Seitz D.E. and Botarro J.C. (1982). *E*-17(α)-[I-125]Iodovinylestradiol: an estrogen-receptor-seeking radiopharmaceutical. *Journal of Nuclear Medicine*, **23**, 431-436.

Harapanhalli R.S., Roy A.M., Adelstein S.J. and Kassis A.I. (1998). [^{125}I/^{127}I/^{131}I]Iodorhodamine: Synthesis, cellular localization, and biodistribution in athymic mice bearing human tumor xenografts and comparison with [^{99m}Tc]hexakis(2-methoxyisobutylisonitrile). *Journal of Medicinal Chemistry*, **41**, 2111-2117.

Hasan A., Allred J.F., Buchsbaum D.J. and Srivastava P.C. (1991). [I-125]iodovinylazomycin acyclonucleosides retain tumor uptake and show low *in vivo* deiodination as compared to [I-125]iodoazomycin acyclonucleoside. *Journal of Nuclear Medicine*, **32**, 1091-1092 [Proceedings].

Hogberg T., Ramsby S., Ogren S-O. and Norinder U. (1987). New selective dopamine D-2 antagonists as antipsychotic agents. *Acta Pharmaceutica Suecica*, **24**, 289-328.

Hutchins R.O. and Taffer I.M. (1983). Aqueous polar aprotic solvents. Efficient sources of nucleophilic oxygen. *Journal of Organic Chemistry*, **48**, 1360-1362.

Iwashina T., Kumar R., Knaus E.E. and Wiebe L.I. (1990). An efficient, high specific activity radioiodination of 5-(1-hydroxy/methoxy-2-iodoethyl)-2'-deoxyuridine by isotope exchange

labelling in pivalic acid melt. *Journal of Labeled Compounds and Radiopharmaceuticals*, **28**, 247-255.

Jerabek P.A., Patrick T.B., Kilbourn M.R., Dischino D.D. and Welch M.J. (1986). Synthesis and biodistribution of ^{18}F-labeled fluoronitroimidazoles: Potential *in vivo* markers of hypoxic tissue. *International Journal of Radiation Applications and Instrumentation Part A. Applied Radiation and Isotopes*, **37**, 599-605.

John C.S., Bowen W.D., Saga T., Kinuya S., Vilner B.J., Baumgold J., Paik C.H., Reba R.C., Neumann R.D., Varma V.M. and McAfee J.G. (1993). A malignant melanoma imaging agent: Synthesis, characterization, in vitro binding and biodistribution of iodine-125-(2-piperidinylaminoethyl)4-iodobenzamide. *Journal of Nuclear Medicine*, **34**, 2169-2175.

John C.S., Bowen W.D., Fisher S.J., Lim B.B., Geyer B.C., Vilner B.J. and Wahl R.L. (1999). Synthesis, *in vitro* pharmacologic characterization, and preclinical evaluation of *N*-[2-(1'-piperidinyl)ethyl]-3-[^{125}I]iodo-4-methoxybenzamide (P[^{125}I]MBA) for imaging breast cancer. *Nuclear Medicine and Biology*, **26**, 377-382.

Jung M.E. and Light L.A. (1982). Preparation of iodoallylic alcohols via hydrostannylation: Spectroscopic proof of structures. *Tetrahedron Letters*, **23**, 3851-3854.

Kabalka G.W., Shoup T.M. and Goodman M.M. (1991). Synthesis of a new series of 17α-iodovinylestradiols via boranes. *Journal of Labeled Compounds and Radiopharmaceuticals*, **30**, 430 [Symposium Abstracts].

Kachur A.V., Dolbier W.R. (Jr.), Evans S.M., Shiue C-Y., Shiue G.G., Skov K.A., Baird I.R., James B.R., Li A-R., Roche A. and Koch C.J. (1999). Synthesis of new hypoxia markers EF1 and [^{18}F]-EF1. *Applied Radiation and Isotopes*, **51**, 643-650.

Kaneta T., Takai Y., Kagaya Y., Yamane Y., Wada H., Yuki M., Iwata R., Tsujitani M., Takahashi S. and Yamada S. (2002). Imaging of ischemic but viable myocardium using a new ^{18}F-labeled 2-nitroimidazole analog, ^{18}F-FRP170. *Journal of Nuclear Medicine*, **43**, 109-116.

Katzenellenbogen J.A., Carlson K.E., Heiman D.F. and Lloyd J.E. (1981). Receptor binding as a basis for radiopharmaceutical design. Radiopharmaceuticals. Structure-Activity Relationships, Edited by R.P. Spencer, Grune & Stratton, Inc., 111 Fifth Avenue, New York, New York, USA, p. 23.

Kiesewetter D.O., Eckelman W.C., Cohen R.M., Finn R.D. and Larson S.M. (1986). Syntheses and D_2 receptor affinities of derivatives of spiperone containing aliphatic halogens. *International Journal of Radiation Applications and Instrumentation Part A. Applied Radiation and Isotopes*, **37**, 1181-1188.

Kiesewetter D.O., Brücke T. and Finn R.D. (1989). Radiochemical synthesis of [^{18}F]fluororaclopride. *International Journal of Radiation Applications and Instrumentation Part A. Applied Radiation and Isotopes*, **40**, 455-460.

Kloster G. and Stöcklin G. (1982). Determination of the rate-determining step in halofatty acid turnover in the heart. *Radioaktive Isotope in Klinik und Forschung*, **15**, 235-241.

Krummeich C., Holschbach M. and Stöcklin G. (1994). Direct n.c.a. electrophilic radioiodination of tyrosine analogues; their *in vivo* stability and brain-uptake in mice. *Applied Radiation and Isotopes*, **45**, 929-935.

Kung H.F., Guo Y.Z., Billings J., Xu X., Mach R.H., Blau M. and Ackerhalt R.E. (1988a). Preparation and biodistribution of [^{125}I]IBZM: A potential CNS D-2 dopamine receptor imaging agent. *Nuclear Medicine and Biology*, **15**, 195-201.

Kung H.F., Kasliwal R., Pan S., Kung M-P., Mach R.H. and Guo Y-Z. (1988b). Dopamine D-2 receptor imaging radiopharmaceuticals: Synthesis, radiolabeling, and in vitro binding of (*R*)-(+)- and (*S*)-(–)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide. *Journal of Medicinal Chemistry*, **31**, 1039-1043.

Kung M-P. and Kung H.F. (1989). Peracetic acid as a superior oxidant for preparation of [^{123}I]IBZM: A potential dopamine D-2 receptor imaging agent. *Journal of Labeled Compounds and Radiopharmaceuticals*, **27**, 691-700.

Kung M-P., Liu B-L., Yang Y-Y., Billings J.J. and Kung H.F. (1991). A kit formulation for preparation of iodine-123-IBZM: A new CNS D-2 dopamine receptor imaging agent. *Journal of Nuclear Medicine*, **32**, 339-342.

Kupfernagel C. (1978). Qualitätskontrolle und Pharmakokinetic ^{11}C-, ^{18}F-, ^{34m}Cl-, ^{77}Br-und ^{123}I-markierter Fettsäuren. *Report Jülich-1551* (Reference in: Coenen *et al.*, 1983).

Lawrence E.O. (1965). Evolution of the cyclotron, Nobel Prize lecture, December 11, 1951. In: Nobel Lectures: Physics, 1922-1941. Elsevier, New York, p. 431.

Lawrence J.H. (1940). Nuclear physics and therapy: preliminary report on a new method for the treatment of leukemia and polycythemia. *Radiology*, **35**, 51.

Leninger A.L. (1982). Principles of Biochemistry, Worth: New York (Reference in: Warren *et al.*, 1990).

Lin C.M., Mihal K.A. and Krueger R.J. (1989). *N*-iodoacetyltyramine: Preparation and use in ^{125}I labeling by alkylation of sulfhydryl groups. *Analytical Biochemistry*, **179**, 389-395.

Lowndes J.M., Hokin-Neaverson M. and Ruoho A.E. (1988). *N*-(3-(*p*-azido-*m*-[^{125}I]iodophenyl)propionyl)-succinimide, a heterobifunctional reagent for the synthesis of radioactive photoaffinity ligands: Synthesis of a carrier-free ^{125}I-labeled cardiac glycoside photoaffinity label. *Analytical Biochemistry*, **168**, 39-47.

Machulla H-J., Marsmann M. and Dutschka K. (1980a). Biochemical concept and synthesis of a radioiodinated phenylfatty acid for in vivo metabolic studies of the myocardium. *European Journal of Nuclear Medicine*, **5**, 171-173.

Machulla H-J., Marsmann M. and Dutschka K. (1980b). Radiopharmaceuticals. I. Synthesis of radioiodinated phenylfatty acids for studying myocardial metabolism. *Journal of Radioanalytical Chemistry*, **56**, 253-261.

Mannan R.H., Somayaji V.V., Lee J., Mercer J.R., Chapman J.D. and Wiebe L.I. (1991). Radioiodinated 1-(5-iodo-5-deoxy-β-D-arabinofuranosyl)-2-nitroimidazole (Iodoazomycin Arabinoside: IAZA): A novel marker of tissue hypoxia. *Journal of Nuclear Medicine*, **32**, 1764-1770.

March J. (1992). Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 4th edition, p. 398.

Mazaitis J.K., Gibson R.E., Komai T., Eckelman W.C., Francis B. and Reba R.C. (1980). Radioiodinated estrogen derivatives. *Journal of Nuclear Medicine*, **21**, 142-146.

McEwan A.J., Wyeth P. and Ackery D. (1986). Radioiodinated iodobenzylguanidine for diagnosis and therapy. *Applied Radiation and Isotopes* **37**, 765-775.

Mennicke E., Holschbach M. and Coenen H.H. (2000). Direct n.c.a. electrophilic radioiodination of deactivated arenes with *N*-Chlorosuccinimide. *Journal of Labeled Compounds and Radiopharmaceuticals*, **43**, 721-737.

Mohammed A., Nicholl C., Titsch U. and Eisenhut M. (1997). Radioiodinated *N*-(alkylaminoalkyl)-substituted 4-methoxy-, 4-hydroxy, and 4-aminobenzamides: Biological investigations for the improvement of melanoma-imaging agents. *Nuclear Medicine and Biology*, **24**, 373-380.

Mulholland G.K., Jung Y-W., Wieland D.M., Kilbourn M.R. and Kuhl D.E. (1993). Synthesis of [^{18}F]fluoroethoxy-benzovesamicol, a radiotracer for cholinergic neurons. *Journal of Labeled Compounds and Radiopharmaceuticals*, **33**, 583-591.

Mulligan R.S., Osman S., Pilowsky L.S., Ell P.J. and Pike V.W. (1999). Preparation of [^{123}I]epidepride from trialkylstannyl precursors in the presence of chloramine-T — Some cautionary observations. *Journal of Labeled Compounds and Radiopharmaceuticals*, **42**, Suppl.1, S774-S776 [Symposium Abstracts].

Musachio J.L. and Lever J.R. (1989). Radioiodination via vinylstannylated alkylating agents. *Tetrahedron Letters*, **30**, 3613-3616.

Musachio J.L. and Lever J.R. (1992). Vinylstannylated alkylating agents as prosthetic groups for radioiodination of small molecules: design, synthesis, and application to iodoallyl analogues of spiperone and diprenorphine. *Bioconjugate Chemistry*, **3**, 167-175.

Napolitano E., Fiáschi R., Carlson K.E. and Katzenellenbogen J.A. (1995). 11β-Substituted estradiol derivatives. 2. Potential carbon-11- and iodine-labeled probes for the estrogen receptor. *Journal of Medicinal Chemistry*, **38**, 2774-2779.

Nicholl C., Mohammed A., Hull W.E., Bubeck B. and Eisenhut M. (1997). Pharmacokinetics of iodine-123-IMBA for melanoma imaging. *Journal of Nuclear Medicine*, **38**, 127-133.

Pfeffer P.E. and Silbert, L.S. (1976). Esterification by alkylation of carboxylate salts. Influence of steric factors and other parameters on reaction rates. *Journal of Organic Chemistry*, 41, 1373-1379.

Pike V.W., Waters S.L., Kensett M.J., Bateman D., Considine D., Turton D.R., Luthra S.K., Brady F., Shah A., Shah F. and Silvester D.J. (1991). Radiopharmaceutical production for PET: Quality assurance practice, experiences and issues. In: New trends in Radiopharmaceutical Synthesis, Quality Assurance, and Regulatory Control, Edited by A.M. Emran, Plenum Press, New York, p. 433-449.

Proescher F. (1914). The intravenous injection of soluble radium salts. *Radium*, **2**, 45.

Rall G.J.H., Oberholzer M.E., Ferreira D. and Roux D.G. (1976). Crown Ethers. Application in the synthesis of chalcones. *Tetrahedron Letters*, 1033-1036.

Rasey J.S., Hofstrand P.D., Chin L.K. and Tewson T.J. (1999). Characterization of [^{18}F]Fluoroetanidazole, a new radiopharmaceutical for detecting tumor hypoxia. *Journal of Nuclear Medicine*, **40**, 1072-1079.

Robinson G.D. (Jr.) and Lee A.W. (1975). Radioiodinated fatty acids for heart imaging: Iodine monochloride addition compared with iodide replacement labeling. *Journal of Nuclear Medicine*, **16**, 17-21.

Saha G.B. (1983). Fundamentals of Nuclear Pharmacy, 2nd edition. Springer Verlag, New York (Reference in: Verbruggen, 1987).

Saji H., Magata Y., Arano Y., Horiuchi K., Torizuka K. and Yokoyama A. (1987). 2-*O*-(*p*-Iodobenzyl)-D-glucose: a new radiopharmaceutical for SPECT studies of cerebral glucose transport. *Journal of Nuclear Medicine*, **28**, 571 [Proceedings].

Seevers R.H. and Counsell R.E. (1982). Radioiodination techniques for small organic molecules. *Chemical Reviews*, **82**, 575-590.

Shah J.H., Kline R.H., Geter-Douglass B., Izenwasser S., Witkin J.M. and Newman A.H. (1996). (±)-3-[4'-(*N*,*N*-Dimethylamino)cinnamyl]benzazepine analogs: Novel dopamine D_1 receptor antagonists. *Journal of Medicinal Chemistry*, **39**, 3423-3428.

Shaw J.E., Kunerth D.C. and Sherry J.J. (1973). A simple, quantitative method for the esterification of carboxylic acids. *Tetrahedron Letters*, 689-692.

Shi B., Narayanan T.K., Yang Z-Y., Christian B.T. and Mukherjee J. (1999). Radiosynthesis and *in vitro* evaluation of 2-(*N*-alkyl-*N*-1'-^{11}C-propyl)amino-5-hydroxytetralin analogs as high affinity agonists for dopamine D-2 receptors. *Nuclear Medicine and Biology*, **26**, 725-735.

Shiue C-Y., Bai L-Q., Teng R. and Wolf A.P. (1986). Application of the nucleophilic substitution reactions to the synthesis of no-carrier-added (nca) ^{18}F-labeled radioligands. *Journal of Labeled Compounds and Radiopharmaceuticals*, **23**, 1038-1039 [Symposium Abstracts].

Srivastava P.C., Lambert C.R., Allred J.F., Kennel S.J. and Hasan A. (1990). I-125 labeled azomycin acyclonucleoside: design and synthesis of an attractive misonidazole analogue for imaging hypoxia. *Journal of Nuclear Medicine*, **31**, 900 [Proceedings].

Srivastava P.C., Hasan A. and Buchsbaum D.J. (1991). Iodoazomycin acyclonucleoside: A potential SPECT imaging agent with high tumor uptake. *Journal of Labeled Compounds and Radiopharmaceuticals*, **30**, 424-425 [Symposium Abstracts].

Stöcklin G., Coenen H.H., Harmand M-F., Kloster G., Knust E.J., Kupfernagel C., Machulla H.J., Weinreich R., Feinendegen L.E., Vyska K., Höck A. and Freundlieb C. (1980). Comparative evaluation of radiohalogenated fatty acids for myocardial and hepatocellular function analysis. *Radioaktive Isotope in Klinik und Forschung* **14**, 151.

Tasker C.W. and Purves C.B. (1949). *p*-Tolylsulfonyl and iodo derivatives of the three lower polyethylene glycol monoethyl ethers. *Journal of the American Chemical Society*, **71**, 1017-1023.

Tewson T.J. (1997). Synthesis of [^{18}F]Fluoroetanidazole: A potential new tracer for imaging hypoxia. *Nuclear Medicine and Biology*, **24**, 755-760.

Tubis M., Krishnamurthy G.T., Endow J.S., Stein R.A., Suwanik R. and Blahd W.H. (1975). Labeled metronidazoles as potential new agents for amebic hepatic abscess imaging. *Nuclearmedizin*, **14**, 163-171.

Vaidyanathan G. and Zalutsky M.R. (1990). Radioiodination of antibodies via *N*-succinimidyl 2,4-dimethoxy-3-(trialkylstannyl)benzoates. *Bioconjugate Chemistry*, **1,** 387-393.

Vaidyanathan G., Affleck D.J., Slade S.A. and Zalutsky M.R. (1995). Labeling of monoclonal antibodies (Mabs) with *N*-Succinimidyl 3-hydroxy-4-[^{131}I]iodobenzoate (*m*SHIB). *Journal of Labeled Compounds and Radiopharmaceuticals*, **37**, 536-538 [Symposium Abstracts].

Van Dort M.E., Kilbourn M.R., Jung Y-W., Sherman P.S. and Wieland D.M. (1999). Synthesis and evaluation of (*E*)-(3-[I-125]iodoprop-2-enyl)ether derivatives of α- and β-

dihydrotetrabenazine as possible radioligands for the neuronal vesicular monoamine transporter (VMAT2). *Journal of Labeled Compounds and Radiopharmaceuticals*, **42**, Suppl.1, S389-S391.

Van Dort M.E. and Hagen C.A. (2001). Synthesis of (*E*)-4-[4,4-dimethyl-2,5-dioxo-3-{1'-(^{125}I)iodo-1'-propen-3'-yl}-1-imidazolidinyl]-2-trifluoromethylbenzonitrile: A potential radioligand for the androgen receptor. *Journal of Labeled Compounds and Radiopharmaceuticals*, **44**, 47-54.

Verbruggen, R.F. (1987). Fast high-yield labelling and quality control of [^{123}I]- and [^{131}I]MIBG. *International Journal of Radiation Applications and Instrumentation Part A. Applied Radiation and Isotopes*, **38**, 303-304.

Visser G. (2002). Radiosyntheses and radiochemical stability evaluation of ^{123}I-labeled organic prosthetic groups linked to model compounds. *B.Tech Thesis*, November 2002, Cape Technikon, Cape Town, South Africa.

Wada H., Iwata R., Ido T. and Takai Y. (2000). Synthesis of 1-[2-[^{18}F]fluoro-1-(hydroxymethyl)-ethoxy]methyl-2-nitroimidazole ([^{18}F]FENI), a potential agent for imaging hypoxic tissues by PET. *Journal of Labeled Compounds and Radiopharmaceuticals*, **43**, 785-793.

Wafelman A.R., Konings M.C.P., Hoefnagel C.A., Maes R.A.A. and Beijnen J.H. (1994). Synthesis, radiolabelling and stability of radioiodinated *m*-iodobenzylguanidine: a review. *Applied Radiation and Isotopes*, **45**, 997-1007.

Wang R.F., Tafani J.A.M., Bergon M., Tisnes P., Coulais Y., Zajac J.M. and Guiraud R. (1995). Synthesis and characterization of 7α-*O*-iodoallyldiprenorphine: A new ligand for

potential SPECT imaging of opioid receptors. *Journal of Labeled Compounds and Radiopharmaceuticals*, **36**, 611-623.

Warren S.D., Ali S.A., Richter K.Y. and Krohn K.A. (1990). Synthesis and radioiodination of ten aryl-carbohydrate compounds for labeling monoclonal antibodies. *Journal of Labeled Compounds and Radiopharmaceuticals*, **28**, 497-509.

Waterhouse R.N., Collier T.L. and O'Brien J.C. (1996). Synthesis of 1-(*trans*-[^{123}I]iodopropen-2-yl)-4-(4-cyanophenoxymethyl)piperidine: A selective sigma receptor radioligand for SPECT. *Journal of Labeled Compounds and Radiopharmaceuticals*, **38**, 215-226.

Waterhouse R.N., Campa M.J., Park J. and Patz, E.F. Jr (1998). Radioiodinated (+)-4-[(αR)-α-[(2*S*,5*R*)-4-(iodopropen-2-yl)-2,5-dimethyl-1-piperazinyl]-3-hydroxybenzyl]-*N*,*N*-diethylbenzamide: A potential ligand for *in vitro* and *in vivo* investigations of δ-opioid receptors. *Journal of Labeled Compounds and Radiopharmaceuticals*, **41**, 801-810.

Weast R.C. (1982). *Handbook of Chemistry and Physics*, CRC-Press Boca Raton, Florida, 63rd edition.

Wester H.J., Herz M., Weber W., Heiss P., Senekowitsch-Schmidtke R., Schwaiger M. and Stöcklin G. (1999). Synthesis and radiopharmacology of *O*-(2-[^{18}F]fluoroethyl)-L-Tyrosine for tumor imaging. *Journal of Nuclear Medicine*, **40**, 205-212.

Wheeler T.N., Craig T.A., Morland R.B. and Ray J.A. (1987). Novel *N*-[4-chloro-2-fluoro-5-(substituted methyl)phenyl]imides. *Synthesis*, 883-887.

Wickham L. and Degrais P. (1911). Traitement des cheloides par le radium. In: *Congres (3) International de Physiotherapie, Paris, 1910, Comptes rendus et communications*. Paris, 1911, 693.

Wilbur D.S., Hadley S.W., Hylarides M.D., Abrams P.G., Beaumier P.A., Morgan C., Reno J.M. and Fritzberg A.R. (1989). Development of a stable radioiodinating reagent to label monoclonal antibodies for radiotherapy of cancer. *Journal of Nuclear Medicine*, **30**, 216-226.

Wilbur S. (1992). Radiohalogenation of proteins: An overview of radionuclides, labeling methods, and reagents for conjugate labeling. *Bioconjugate Chemistry*, **3**, 433-470.

Wilson A.A., Dannals R.F., Ravert H.T., Burns H.D. and Wagner H.N., Jr. (1986). I-125 and I-123 labeled iodobenzyl bromide, a useful alkylating agent for radiolabelling biologically important molecules. *Journal of Labeled Compounds and Radiopharmaceuticals*, **23**, 83-93.

Windhorst A.D., Timmerman H., Menge W.M.P.B., Leurs R. and J.D.M. Herscheid (1999). Synthesis, *in vitro* pharmacology and radiosynthesis of *N*-(*cis*-4-fluoromethylcyclohexyl)-4-(1(H)-imidazol-4-yl)piperidine-1-thiocarbonamide (VUF 5000), a potential PET ligand for the histamine H_3 receptor. *Journal of Labeled Compounds and Radiopharmaceuticals*, **42**, 293-307.

Yasunaga T., Kimura T., Naito R., Kontani T., Wanibuchi F., Yamashita H., Nomura T., Tsukamoto S., Yamaguchi T. and Mase T. (1998). Synthesis and pharmacological characterization of novel 6-fluorochroman derivatives as potential 5-HT_{1A} receptor antagonists. *Journal of Medicinal Chemistry*, **41**, 2765-2778.

Zielinski F.W., Holly F.E., Robinson G.D. and Bennett L.R. (1977). Total and individual kidney function assessment with Iodine-123 ortho-iodohippurate. *Radiology*, **125**, 753-759.

Zijlstra S., Elsinga P.H., Oosterhuis E.Z., Visser G.M., Korf J. and Vaalburg W. (1993a). Synthesis and *in vivo* distribution in the rat of several fluorine-18 labeled 5-hydroxy-2-aminotetralin derivatives. *Applied Radiation and Isotopes*, **44**, 473-480.

Zijlstra S., Visser G.M., Korf J. and Vaalburg W. (1993b). Synthesis and *in vivo* distribution in the rat of several fluorine-18 labeled *N*-fluoroalkylapomorphines. *Applied Radiation and Isotopes*, **44**, 651-658.